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PEPTIDE AGGREGATION IN NEURODEGENERATIVE DISEASE

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■ **Abstract** In the not-so-distant past, insoluble aggregated protein was considered as uninteresting and bothersome as yesterday's trash. More recently, protein aggregates have enjoyed considerable scientific interest, as it has become clear that these aggregates play key roles in many diseases. In this review, we focus attention on three polypeptides: beta-amyloid, prion, and huntingtin, which are linked to three feared neurodegenerative diseases: Alzheimer's, "mad cow," and Huntington's disease, respectively. These proteins lack any significant primary sequence homology, yet their aggregates possess very similar features, specifically, high β-sheet content, fibrillar morphology, relative insolubility, and protease resistance. Because the aggregates are noncrystalline, secrets of their structure at nanometer resolution are only slowly yielding to X-ray diffraction, solid-state NMR, and other techniques. Besides structure, the aggregates may possess similar pathways of assembly. Two alternative assembly pathways have been proposed: the nucleation-elongation and the template-assisted mode. These two modes may be complementary, not mutually exclusive. Strategies for interfering with aggregation, which may provide novel therapeutic approaches, are under development. The structural similarities between protein aggregates of dissimilar origin suggest that therapeutic strategies successful against one disease may have broad utility in others.

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INTRODUCTION

Amyloid is a general term describing protein aggregates with several physicochemical features in common: a fibrillar morphology, a predominantly β-sheet secondary structure, birefrigence upon staining with the dye Congo red, insolubility in common solvents and detergents, and protease-resistance. Huntington's, Alzheimer's, and spongiform encephalopathy diseases are neurodegenerative disorders that have in common the presence of insoluble protein aggregates near the site of disease. Characteristic of Alzheimer's disease are senile plaques, extracellular deposits of beta-amyloid peptide fibrils surrounded by degenerating neurites. Spongiform encephalopathies include scrapie, "mad cow," and Creutzfeld-Jacob disease. Deposits of aggregated prion protein, some of which have the structural features of amyloid, are observed in brain tissues from humans and animals with these related diseases. Huntington's disease is characterized by insoluble aggregates of an N-terminal fragment of the protein huntingtin; these aggregates are intraneuronal inclusions localized to the nucleus. At least some of these huntingtin-containing nuclear inclusions stain with Congo red, and fibrillar structures have been observed, indicating that huntingtin aggregates may also be classified as amyloid-like (1).

None of the three polypeptides implicated in these diseases shares any primary sequence homology (Figure 1*A*), nor do they derive from similar sources. Beta-amyloid peptide (Aβ) is a small ($\sim$4 kDa) proteolytic cleavage product of the $\sim$70-kDa transmembrane protein APP (amyloid precursor protein) (2). Huntington's disease is causally linked to an expanded polyglutamine repeat domain (>35 glutamines) in the N-terminal region of the huntingtin protein (htt). Huntingtin is a 350-kDa protein of unknown function localized in the cytoplasm; release of the N-terminal fragment by proteolytic cleavage seems to be required to initiate aggregation and transfer to the nucleus (3, 4). The prion protein (PrP) is a glycosylphosphatidylinositol-anchored glycoprotein ($\sim$34 kDa) that is a normal cell-surface component of neurons. No proteolysis or covalent modification appears to be required to initiate aggregation (5). Each aggregate has unique clinical manifestations, likely due to localized effects on specific subsets of neurons. Only the prion protein appears to be capable of transmitting disease.

Still, there are striking parallels (and differences) in the physicochemical properties of these three diverse polypeptides. Many detailed biophysical studies have been published, using both chemically synthesized peptides and recombinant proteins. Key experimental techniques include circular dichroism, FTIR, and NMR

A.

Protein fragment	Primary sequence (1-letter amino acid code) and predicted secondary sequence
Aβ(1-40)	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV coil helix/sheet coil β-sheet
PrP(106-126)	KTNMHKHMAGAAAAGAVVGGLG coil α-helix β-sheet coil
Htt(1-17-Q_n)	MATLEKLMKAFESLKSFQQQQQQQQQQQQQQQQQQ coil α-helix helix/coil coil

B.

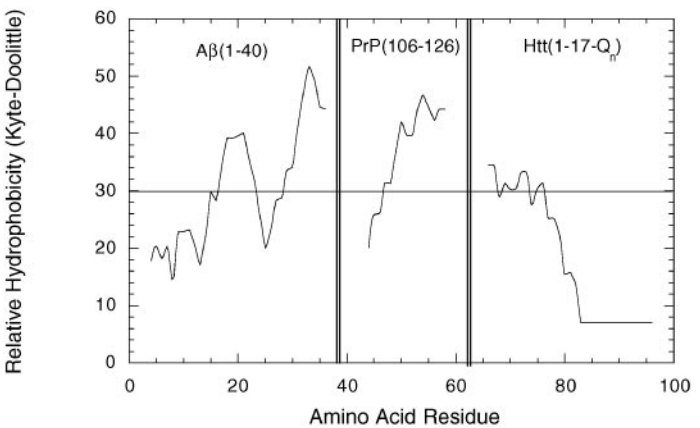

Figure 1 Three aggregating polypeptides related to neurodenegerative disease. (*A*) Primary sequences of beta-amyloid Aβ[1–40], human prion peptide PrP[106–126], and N-terminal huntingtin (htt) fragments. Primary sequences are taken from the Brookhaven Protein Data Bank. Full-length PrP and longer N-terminal huntingtin fragments are found in tissue deposits. The secondary structure predictions are based on a consensus of eight algorithms available in the Biology Workbench. Regions of disagreement between alternative algorithms are indicated as a choice of two structures. (*B*) Kyte-Doolittle hydropathy profiles. Residues below the dotted line are characterized as having hydrophilic side chains; residues above the dotted line are considered as having hydrophobic side chains.

spectroscopies; electron and atomic force microscopy; X-ray diffraction; analytical ultracentrifugation; size-exclusion chromatography; and light scattering. In this brief review, we discuss recent efforts (*a*) to elucidate the structure of these polypeptides in both soluble and aggregated states, (*b*) to define the kinetics of conversion of monomer to aggregate, and (*c*) to discover compounds capable of interfering with polypeptide aggregation. Such compounds may serve as leads in the effort to develop effective therapies against these devastating neurodegenerative diseases.

SOLUTION STRUCTURES OF SELF-ASSEMBLING POLYPEPTIDES

An interesting hypothesis is that peptides prone toward amyloid fibril formation are those that as monomers fold into α-helices in domains that are predicted to be β-sheet (6). To test this hypothesis against the three peptides under discussion, eight widely available secondary-structure prediction algorithms were used to analyze the peptide fragment sequences shown in Figure 1*A*. These algorithms were developed for globular soluble proteins, and their extension to the three polypeptides of interest here is problematic. With this caveat in mind, we report on the consensus sequence. All three peptides are predicted to have a disordered N-terminus. The C-terminus of Aβ[1–40] (residues 1 through 40 of Aβ) is invariably predicted to be β-sheet, but the interior region (residues 11–21) is a domain of conformational confusion: The algorithms split about equally between helix or sheet. PrP[106–126] is predicted to contain an interior helix followed by a short β-strand. The huntingtin fragment is predicted to be primarily α-helical. Based on these three peptide sequences, there is no consistent predicted propensity towards β-sheet, and hence, no support for the hypothesis. How well do these analyses compare to experimental data? The solution structures of monomeric polypeptides related to Aβ, PrP, and htt have been reported by a number of investigators. A general feature is the conformational flexibility or adaptability of these polypeptides. In fact, proteins unrelated to known disease states can be induced to form amyloid fibrils by reducing the conformational stability of the folded globular protein (7). Perhaps a modified version of the hypothesis is more globally applicable; specifically, proteins and peptides prone to amyloid fibril formation are those that have a domain that readily adopts multiple conformations.

In Figure 1*B* we compare the hydrophobicity profiles of the three peptides, calculated using the Kyte-Doolittle method. The profiles of Aβ[1–40] and PrP[106–126] are remarkably similar: Both are amphiphilic, with a hydrophilic N-terminus and hydrophobic C-terminus. The origins of these peptides have an impact on their hydrophobicity profile: Both Aβ and the prion protein originate as membrane-embedded proteins. In fact, cleavage at the C-terminus of Aβ occurs in the interior of the transmembrane domain. It is reasonable to hypothesize that their hydrophobic character plays a considerable role in the peptides' aggregation properties. Huntingtin is quite distinct. Full-length htt is cytoplasmic, not membrane derived

(although, interestingly, the aggregates are found not in the cytoplasm but in the nucleus). The polyglutamine expansion domain, strongly linked to aggregation and to disease, is quite hydrophilic on the Kyte-Doolittle scale. This analysis may be somewhat misleading; polyglutamine may act like a much more hydrophobic group due to strong hydrogen bonding between the polypeptide backbone and side chain amides. The distribution of hydrophobic side chains likely plays a significant role as well. It is possible to generate libraries of synthetic peptides of alternating polar and nonpolar residues that have a strong predilection towards self-associating into amyloid-like aggregates (8).

Several groups employed circular dichroism and NMR spectroscopies to explore solution-phase secondary structure of Aβ-, PrP- and htt-related peptides. Results are summarized briefly in Table 1; each peptide is discussed in some detail in the following sections.

Aβ

Aβ undergoes substantial conformational shifts depending on its environment and can easily convert among disordered, α-helical, and β-sheet conformers as solution conditions change. Under membrane-mimicking conditions, Aβ contains a significant amount of α-helical character (9–11). In physiological buffers, both random coil and β-sheet secondary structure are observed, with the β-sheet content increasing dramatically with peptide concentration (9). This indicates that the β-sheet-containing conformers are oligomeric. Aβ conformation is both pH- and salt-sensitive, with an increase in β-sheet content in the presence of salt and in slightly acidic conditions. His residues at positions 12 and 13 likely contribute to the pH effect on the stability of aggregates, while the increase in β-sheet with salt likely derives from the peptide's hydrophobic regions (12, 13). NMR solution studies on Aβ[10–35] indicated that the soluble monomer in water lacks regular secondary structural features (14). Rather, the peptide adopts a meta-stable collapsed coil structure around the central hydrophobic region (residues 17–21), with a large hydrophobic patch on the surface, and a turn in the 24–27 region (14).

TABLE 1 Key secondary structural features of neurodegeneration-related aggregating peptides

	Aβ	**PrP**	**htt**
pH-dependence	β-sheet ↑ with slightly acidic conditions	β-sheet ↑ with slightly acidic conditions	—
Salt-dependence	β-sheet ↑ with ↑ salt	β-sheet ↑ with ↑ salt	—
Concentration-dependence	β-sheet ↑ with ↑ concentration	β-sheet ↑ with ↑ concentration	—
In membrane-mimicking solvents	α-helix	—	β-sheet

A molecular dynamics study of Aβ[10–35] noted structural fluctuations outside of the core hydrophobic (residues 17–21) domain and the turn (residues 24–27) region (15). The picture that emerges is of a peptide that adopts a helical structure when anchored in its natural membrane environment, that undergoes hydrophobic-driven collapse into a monomer lacking regular secondary structural features when released from the membrane by proteolysis, and that subsequently simultaneously oligomerizes and forms an extended β-sheet.

PrP

The solution structure of PrP[106–126] in water is predominantly random coil with some α-helical character (16), but reverts to β-sheet upon addition of a physiological concentration of salt (17). PrP fragments exhibit high conformational flexibility; addition of just five residues N-terminally to PrP[109–122] converts the stable conformer from β-sheet to α-helix (18). The solution structure of full-length recombinant PrP has been determined by NMR. The N-terminus (residues 1–125, roughly half of the protein) has a long flexible tail; the C-terminal globular domain contains three α-helices and a short anti-parallel β-sheet region (14, 19). Within the globular domain there is a flexible loop (residues 167–171) connecting the second β-strand and the second helix. C-terminal portions of helices 2 and 3 have relatively large conformational flexibility (14); indeed, synthetic peptides corresponding to these regions underwent time- and pH-dependent conversion to β-sheet aggregates (20). Under normal conditions, the solution structure of full-length PrP is quite stable; however, incubation of the protein under acidic conditions and in the presence of salt and low concentrations of denaturant causes a reproducible conversion to β-sheet oligomers (21). The His residue, lying between the hydrophilic N-terminus and hydrophobic C-terminus of PrP[106–126], may play a crucial role in the pH-dependence of aggregation and conformational change (22). Hydrogen exchange studies demonstrated that the conformational stability of the helical region of monomer PrP is not substantially different than other similar proteins (23). Removal of the single disulfide bond substantially reduces the stability of the helical monomer (24). Reduction of the disulfide bond, and acidic pH, caused a slow but reversible conformational shift in PrP[91–231] from helix to β-sheet; rather suprisingly, the β-sheet conformer was monomeric, stable, and completely soluble (25). Molecular dynamic simulations of PrP[121–231] (26) suggested formation of a hydrophobic cluster involving a short-lived addition of a third β-strand involving residues 123–125 to the antiparallel β-strands present in the stable folded prion monomer; perhaps this structural fluctuation initiates conversion from the predominantly helical monomer to the β-sheet aggregate (26). This picture is in agreement with the hypothesis, based on X-ray diffraction studies, that the central hydrophobic region of PrP forms a core that facilitates conversion of helical PrP to β-sheet (27). Taken together, these reports suggest that the native helical folded structure of monomeric PrP is only marginally stable, that short β-strands positioned near a hydrophobic core initiate conformation fluctuations, and that, by undergoing association, a β-sheet structure of greater stability can be obtained.

Huntingtin

Very few detailed structural studies have been completed on polyglutamine or polyglutamine-containing htt fragments, in part because of difficulties in synthesis and insolubility. Based on circular dichroism studies, polyglutamines are strong β-sheet formers, retaining a β-sheet structure even in the helical-promoting solvent trifluoroethanol (28). The β-sheets are stabilized by hydrogen bonding between main-chain and side-chain amides. Indirect evidence suggested that long (40-mer) polyglutamine peptides can form stable μ-helices, a novel tubular single-stranded helix with an inner wall containing a network of peptide backbone hydrogen bonds (29).

Thus, there are many parallels between PrP and Aβ monomer structures in aqueous solution: significant regions of disorder and conformational flexibility, a hydrophobic cluster with β-sheet-forming tendencies, and His-mediated pH sensitivity. Full-length PrP is more stable as monomer than is Aβ, likely because the former folds into a monomer with significant regular secondary structure whereas the latter does not. The difference is one of degrees, though, rather than of kind. Huntingtin, however, is a different kind of peptide. This was observed in the comparative analyses of the primary sequences (Figure 1*A* and 1*B*), and in the stability of the β-sheet in α-helix promoting solvents (Table 1). Further structural studies of htt vis-a-vis Aβ and PrP are needed to tease out a generalizable relationship between sequence, structure, and amyloidogenesis.

SOLID-STATE STRUCTURES OF SELF-ASSEMBLED POLYPEPTIDES

Substantial conformational changes occur upon the conversion of soluble peptide to insoluble aggregate. Ascertaining the structure of the aggregates has proved to be a challenge. High resolution structural analysis of the aggregates has proven difficult to date because the fibrils are noncrystalline and do not provide clear NMR signals without special labeling techniques. Still, some advances have been made in ascertaining structure of the aggregates, especially for Aβ aggregates. Useful techniques include electron and atomic force microscopy, X-ray diffraction, and solid-state NMR.

Aβ

Long, semi-flexible, nonbranching fibrils of ~6–10 nm diameter are visible on electron microscope images of Aβ aggregates. Cross-sectional analysis of electron microscope images of aggregated Aβ (30) imputed a structural model of amyloid fibrils as an assembly of three to six laterally associated filaments. More detailed structural information was obtained in several studies employing atomic force microscopy. These studies yielded images of "protofilaments," thin (3–4 nm) diameter nonbranching linear aggregates (31, 32). Growth of the filaments

proceeded bidirectionally (33). Protofilaments were indirectly observed by X-ray diffraction as well (34). These studies indicate that lateral association of several protofilaments produces the larger-diameter amyloid fibrils.

There are striking differences in conformation between Aβ monomer and aggregate. In contrast to the conformational flexibility of the monomer, about half of the amide protons in multimeric Aβ fibrils are highly-resistant to solvent exchange (35), indicating the core is highly stable. The central core region of Aβ (residues 14–23) is competent to form fibrils, and deletion of this core from Aβ[1–42] abrogates fibril formation (36). X-ray diffraction studies combined with molecular modeling produced a detailed structure of Aβ[11–25] fibrils: a β-hairpin with a turn at residues 18–19, an antiparallel arrangement of β-strands perpendicular to the long axis of the fiber, forming continuous β-sheets, and inter-sheet interactions forming the filaments (37). These studies led to the proposal that Aβ aggregates are composed of a highly-stable anti-parallel β-sheet core containing residues 14–23, with the hydrophobic C-terminus folding over this core. However, the applicability of this structural model to full-length Aβ has been challenged by more recent solid-state NMR studies. A detailed examination of Aβ[10–35] fibrils produced the surprising result that Aβ forms parallel β-sheets, with no turns and with like residues in-register (38). Multiple-quantum solid-state NMR on full-length Aβ[1–40] further supported a parallel, in-register arrangement of Aβ monomers in the fibrils, with the parallel arrangement extending over at least four peptide chains (39).

PrP

In vitro, many PrP fragments readily form amyloid-like aggregates. Electron micrograph images of PrP[106–126] and PrP[178–193] aggregates reveal the characteristic fibrillar nonbranching morphology of amyloid (20, 40). Similarly, PrP[90–231] can be induced, under partially denaturing conditions, to form both amorphous and fibrillar structures (21, 41). As observed in X-ray diffraction studies, PrP fragments with single-point mutations readily formed thin (4-nm diameter) fibrils with cross-β sheet structure (27); hydrated wild-type fragments only infrequently folded into β-sheets. These authors proposed that the hydrophobic [106–126] domain serves as a core that facilitates conversion of the full-length PrP to β-sheet. This hypothesis is consistent with other reports showing that the hydrophobicity of the PrP fragment plays an important role in facilitating β-sheet formation and aggregation (42). In vitro, full-length PrP forms β-sheet aggregates after mild denaturation under slightly acidic conditions (21). Together, these studies indicate that PrP fragments form amyloid aggregates with physical properties very similar to those of Aβ. The biological relevance of these in vitro studies has been questioned; in animals with scrapie, for example, amyloid aggregates are not always observed. This is in sharp contrast to the case with Aβ, since the presence of Aβ amyloid deposits is one of the defining features of Alzheimer's disease.

Huntingtin

N-terminal huntingtin fragment htt(1-90) with 37 or more glutamines produced almost exclusively SDS-insoluble high-molecular-weight aggregates (43); by electron microscopy, aggregates had the classical fibrillar morphology, 100 to >1 μm in length. Polyglutamines aggregate into semiflexible chains of 7–12-nm diameter; X-ray diffraction patterns are consistent with the cross-β structure characteristic of amyloid fibrils (28). These reports demonstrate that peptides with long polyglutamine stretches can be induced to form amyloid aggregates in vitro. There remains a great deal to learn about the ultrastructure of htt aggregates. As with PrP, the biological relevance of in vitro htt amyloid aggregates has been questioned. There is limited evidence that the in vivo nuclear inclusions have amyloid-like properties (1).

KINETICS OF POLYPEPTIDE SELF-ASSEMBLY

Elucidation of the kinetic pathway by which monomer is converted to fibril has occupied the attention of several research groups. Broadly speaking, there are two schools of thought: the nucleation-elongation model and the template-assisted model. In the nucleation-elongation model, initial self-assembly is slow and unfavorable until a critical size is reached. Once the "nucleus" is formed, further elongation by addition of monomers is rapid. In the template-assisted model, exposure of the monomer to an aggregate catalyzes a conformational change of the non-β monomer into a β-rich form that is aggregation prone. Roughly equivalent to the nucleation-elongation versus template-assisted models, one can consider whether peptide self-assembly is spontaneous (does not require existence of pre-existing aggregate) or induced. Experimental and modeling efforts are briefly reviewed to see what evidence exists for either model of peptide assembly.

Spontaneous conversion of soluble Aβ monomer to amyloid is easily achieved in neutral or slightly acidic buffers containing physiologically relevant salt concentrations. Using turbidity to measure Aβ aggregation, Jarrett et al. (44) observed a concentration-dependent lag time in the appearance of aggregates and proposed a qualitative nucleation-elongation kinetic model for Aβ self-association. However, several other studies indicated that aggregates too small to be detectable by turbidity are present early in the aggregation process (45, 46). Thus, the lag phase observed in turbidity assays is likely not the time required for nucleation, but rather the time required for growth of sufficiently large aggregates. Lomakin et al. (47) employed dynamic light scattering to study fibril growth from Aβ[1–40] in 0.1 M HCl and proposed a detailed mathematical model based on these data along the lines of the nucleation-elongation hypothesis. Briefly, rapid reversible equilibration between monomers and micelles was postulated to occur, followed by spontaneous and irreversible generation of nuclei from micelles. Fibrils then grew by addition of monomer to the nucleus or fibril tip. A conceptually similar model was proposed by Inouye & Kirschner (48) in which association of multiple

monomers into a nucleus precedes indefinite reversible addition of monomers to polymer. This model was used to describe the pH-dependent growth of Aβ aggregates, as monitored by Congo red binding. A detailed multi-step model of Aβ aggregation kinetics was also proposed by Pallitto & Murphy (49); a schematic is shown in Figure 2. This pathway included: (*a*) rapid commitment to either stable monomer/dimer or unstable β-sheet intermediate, (*b*) cooperative association of intermediate into a multimeric "nucleus," (*c*) elongation of the "nucleus" into

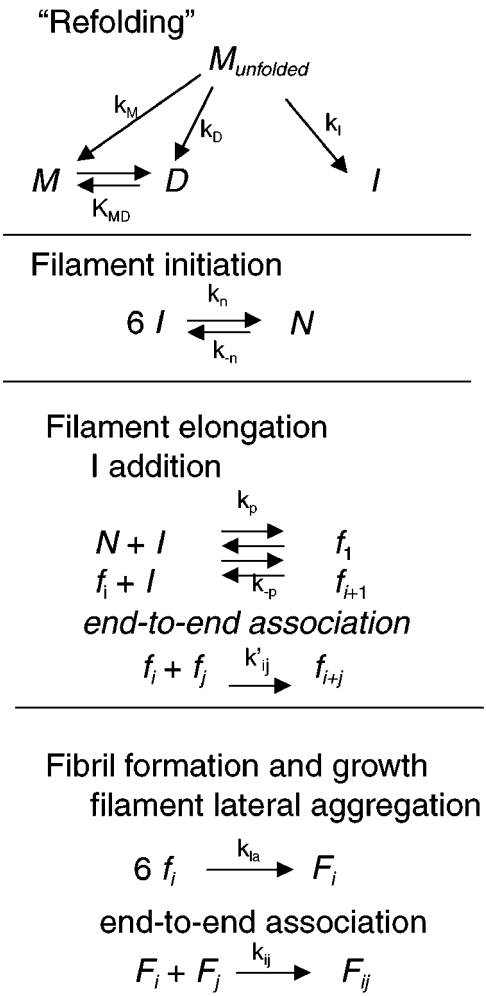

Figure 2 Schematic of solution-phase self-association of Aβ[1–40], adapted from (49). A mathematical model quantifying this schematic was derived and fitted using experimental data. Qualitatively similar pathways may be appropriate for aggregation of PrP and, possibly, htt.

filaments via addition of intermediate, (*d*) lateral aggregation of filaments into fibrils, and (*e*) fibril elongation via end-to-end association. The model was shown to be consistent with several sets of complementary experimental data (49). All of these models argue for the existence of a spontaneous mode of Aβ self-assembly.

As mentioned above, PrP monomers are more stable than Aβ in aqueous solution and conversion to aggregates is more difficult. Aggregation of PrP[106–126], as measured by turbidity, followed the classic sigmoidal curve observed with Aβ (42). These data were interpreted as supportive of the nucleation-condensation model, but it could be that aggregates present during the early lag phase were not detectable by turbidity. Acidic pH and addition of low or moderate concentrations of a chemical denaturant were used to achieve conversion of the "native" structure of human recombinant PrP[90–231] (disordered N-terminus, helical C-terminus) to β-sheet. Conversion of PrP to β-sheet structure was invariably coincident with oligomerization (21, 24). Post et al. studied conformational changes and oligomerization with PrP[27–30] and PrP[90–231] solubilized with SDS. Once removed from the α-helical stabilizing detergent solution, a rapid ($<$1 minute) conversion to β-sheet dimers was observed, followed by the appearance of larger oligomers after 20 minutes and the appearance of protease-resistant aggregates after several hours (50). Dimer to multimer conversion did not appear to require a cooperative step. Hydrogen-exchange studies demonstrated that conversion of helical PrP to β-sheet must proceed through a partially or completely unfolded intermediate (23). Urea-denatured PrP refolded to either its monomeric, predominantly helical form, or to its aggregation-prone, predominantly β-sheet form (41); the α-helical form was kinetically favored, but the β-sheet form was thermodynamically more stable. The kinetics of refolding of the C-terminal region of PrP proceed at an extremely rapid pace (51), in further support of the hypothesis that PrP conversion does not occur through a populated folding intermediate state. Taken together, the data indicate that denatured PrP refolds into two alternative structures, one a stable folded helical monomer and the other an unstable aggregation-prone β-sheet-rich conformer. Although the quantitative details differ between the two peptides, the kinetic pathway shown in Figure 2, which was developed for Aβ, captures the essential features of these data on PrP assembly.

Conversion of htt from monomer to aggregate depends strongly on the length of the polyglutamine domain. htt[1–90] with 32 or fewer glutamines was monomeric, but htt[1–90] with 37 or more glutamines produced almost exclusively SDS-insoluble high-molecular-weight aggregates with a fibrillar morphology (43). Using a turbidity assay, these researchers observed a lag-time in the onset of turbidity, with the length of the lag decreasing with increasing number of glutamines in the expansion region and increasing concentration. Addition of preformed fibrils eliminated the lag time (43). These studies led the authors to propose that htt aggregation proceeded via a nucleation-elongation mode. Due to the relative paucity of kinetic data on huntingtin aggregation, we cannot yet speculate whether htt association kinetics follow the pathway outlined in Figure 2. If the polyglutamine expansion domain is sufficiently long, the peptide can fold into a stable β-sheet monomer with

a hairpin turn (28). This result indicates that, unlike Aβ or PrP, htt monomer with an expanded polyglutamine region may not require re-folding from the completely unfolded state to initiate slow aggregation.

A lattice-type model of protein folding and β-sheet propagation has been proposed and applied to the generation of PrP or Aβ aggregates (52). Interesting conclusions from this study included (*a*) efficient propagation requires two opposite-facing binding sides, and (*b*) the most readily propagatable conformation is β-sheet rich. The hydrophobic nature of the peptides, or capacity for hydrogen bonding, was not explicitly considered. Perhaps any kind of oppositely faced attractive interaction can lead to propagation, which might explain why htt fragments, despite their hydrophilicity, can aggregate via backbone–side chain hydrogen bonding (28).

In the previous paragraphs, we discussed the kinetics of spontaneous conversion of solution-phase peptide to fibrillar aggregates. We now turn attention to induced conversion of solution-phase peptide to β-sheet aggregates. Roughly speaking, the difference between spontaneous and induced conversion parallels the difference between the nucleation-elongation and template-assisted models. Several lines of evidence indicate that template-assisted conversion is an important phenomenon in the peptide systems under discussion.

When pre-assembled Aβ fibrils were immobilized onto a solid surface, monomeric, not oligomeric, Aβ bound to the fibrils (53) via a "dock-lock" mechanism in which weak reversible binding of monomer to fibril was followed by a conformational change "locking" the monomer to the template (54). In a clever study, Esler et al. synthesized a highly constrained structural analog of soluble Aβ and an analog that should more easily undergo conformational changes than wild-type Aβ; deposition rates were strongly dependent on conformational flexibility (55). These data support a template-assisted mechanism of growth, and suggest that the kinetic limit to deposition is the conformational transition of the soluble species. A strong argument has been made that much of the data on prion infectivity fits the template-assisted model (56). Interestingly, a prion peptide fragment PrP[109–122] was capable of converting normally α-helical peptide fragments into β-sheet conformers (18). Similarly, proteins with extended polyglutamine regions can "recruit" proteins with normal-length polyglutamine stretches into the aggregates, even when the normal length polyglutamine proteins would not aggregate by themselves; recruitment depends on interactions between the polyglutamine domains (57). These studies further support the existence of a mechanism for induced conversion of monomer peptide to β-sheet aggregate.

Most likely, both spontaneous and induced conversion of monomers of Aβ, PrP, and htt to β-sheet aggregates occur. The relative importance of the two alternative modes depends on the conformational stability of the peptide monomer, peptide concentration, the presence of pre-existing aggregated material, and the mode of presentation of aggregated material to soluble monomer (e.g., suspended or immobilized aggregates).

In one interesting model, the authors skirted the issue of kinetics of conversion and looked at the kinetics of pathogenesis, considering the kinetics of amyloid production, metabolism, and cell-to-cell transport (58). Another model postulated a mechanism by which interspecies transmission of prion disease may occur. Briefly, the formation of an intermediate conformational state from host cellular PrP (PrP^C) was postulated to be catalyzed by inoculation with heterologous scrapie-form PrP (PrP^{Sc}), with conversion of host PrP^C to host PrP^{Sc} catalyzed both by this intermediate and autocatalytically by PrP^{Sc} (59). Although interesting, these studies must be considered speculative rather than definitive, as many of the model parameters and even the model structure were not rigorously verified by experiment.

ARE THE AGGREGATES TOXIC?

The conventional view has been that amyloid aggregates are pathological. Numerous studies have shown that aggregated Aβ is toxic in vitro (60, 61), and toxicity has been linked to a specific fibrillar morphology (62). More recently, however, an alternative view is emerging: that it is not the insoluble Aβ aggregates themselves, but rather an oligomeric intermediate that is the primary toxic species (49, 63–65).

In vitro toxicity of several PrP fragments has been demonstrated (66). The toxicity correlated with hydrophobicity of the core AGAAAAGA sequence (42, 67), rather than specifically β-sheet structure or fibrillar morphology of aggregates (67). No obligatory correlation between formation of aggregates with amyloid properties (e.g., Congo red binding, fibril morphology, protease resistance) and infectivity of scrapie prion protein fragments was observed, but the β-sheet content did correlate with infectivity (68). This result is consistent with the hypothesis that, for PrP also, oligomeric β-sheet intermediates, rather than the insoluble aggregates, are required for infectivity and/or toxicity.

There is considerable disagreement in the literature as to whether aggregates in Huntington's disease are directly toxic. Li et al. observed a strong correlation in mutant mice between production of N-terminal huntingtin fragments, aggregation, and selective neuritic degeneration (69). A different conclusion was reached in another study in which it appeared that there was no correlation between huntingtin aggregates and cell loss; in fact, it was suggested that aggregates serve a cytoprotective role (70). Inhibition of caspase reduced generation of huntingtin fragments, extent of aggregation, and toxicity (71). Intracellular deposits of aggregated htt with expanded polyglutamine domains directly inhibited normal functioning of the ubiquitin-proteasome system, an effect that could lead to cellular dysfunction and death (72). Hsp70 suppressed polyglutamine toxicity without a visible effect on aggregate formation in a fruit-fly model (73). A different conclusion came out of a study employing mammalian cells transfected with the gene encoding for the huntingtin fragment; both GroEl and Hsp104 expression reduced polyglutamine-mediated aggregation and cell toxicity (74). This remains a controversial issue [see Ref. (75) for a brief review].

INHIBITORS OF AGGREGATION AND/OR TOXICITY

Because aggregates are associated with pathology, efforts are underway to develop compounds that interfere with aggregation of htt, PrP, and Aβ, with the hope that such compounds will also prevent toxicity. Interestingly, several compounds have turned up as potentially useful against more than one of these chemically distinct peptides.

One class of promising candidates for interference of self-assembly of neuropeptides includes the sulfonated dyes Congo red and thioflavine S, both of which are used as histochemical stains for amyloid fibrils. Congo red disrupts Aβ aggregation and toxicity (76, 77) and inhibits fibrillogenesis of huntingtin fragments (78). Chrysamine G, a more lipophilic variant of Congo red, was also effective against Aβ (79) and huntingtin (78). Several other small molecules, typically with highly conjugated cyclic groups, have been successful to different degrees as inhibitory compounds. Daunomycin and related anthracyclines, rifampicin and related naphthahydroquinones, and benzofurans reportedly interfered with Aβ aggregation and/or toxicity (80, 81). Porphyrins and phthalocyanines inhibited conversion of soluble PrP to its protease-resistant form independent of charge group (82). From a large library of imidazopyridoindoles, some compounds active against Aβ were found; these compounds inhibited random coil to β-sheet conformational transition, inhibited aggregation, and prevented neurtoxocity (83). One interesting compound is a pyridone that enhances aggregation of both Aβ and PrP fragments (84).

Another approach for inhibiting aggregate formation that has met with some success is the use of specific antibodies targeted against the peptide domain assumed to be essential for aggregation. Nuclear inclusion formation in cells was greatly reduced by coexpression of a huntingtin fragment and a single-chain Fv antibody targeted to the N-terminus of huntingtin (85). In in vitro studes, an antibody that recognizes only the soluble form of extended polyglutamine domains of proteins inhibited fibril formation, although significant quantities of amorphous aggregated materials were detected (78). Antibodies raised against the N-terminus of Aβ prevented fibril formation in vitro, partially restored peptide solubility of preformed Aβ fibrils, and inhibited toxicity (86).

Because the peptides under discussion are self-assembling, it may be possible to target each peptide specifically by using a short peptide fragment homologous to a segment of the full-length peptide. This idea has occurred to several groups, and implementation of the idea has met with some success. Of particular interest are those peptide-based compounds that, by binding to the self-assembling peptide, interfere with its assembly into (presumably) toxic aggregates. Furthest advanced are studies with peptide-based inhibitors of Aβ. The sequence KLVFF, corresponding to residues 16–20 of Aβ (the "conformationally confused" region; Figure 1*B*) was one of the most effective pentapeptides in binding to and inhibiting Aβ aggregation (87). Several variations on this theme have been investigated with some success. Substitution of prolines for some of these residues produced "β-sheet breaker" peptides reportedly capable of inhibiting Aβ aggregation and

toxicity (88). N-methylated Aβ[25–35] peptides were able to inhibit toxicity of Aβ, possibly by binding to Aβ and preventing further intermolecular hydrogen bonding (89). In a slightly different approach, attachment of nonhomologous peptide sequences or other groups to the KLVFF sequence produced compounds capable of inhibiting Aβ aggregation and/or toxicity (90–92). Some of the peptidyl compounds most effective at inhibiting Aβ toxicity actually accelerate the Aβ aggregation rate (93). This unexpected result is in line with the hypothesis that intermediates in the aggregation pathway, not the end products themselves, are the toxic moiety.

Taking a similar approach, several short peptides homologous to the central and C-terminal regions of PrP have been shown to be effective at inhibiting conversion of soluble PrP to the β-sheet-rich protease-resistant form (94, 95) or at inhibiting PrP toxicity in vitro (67). The mechanism of action appears to involve binding of the peptide inhibitor to the soluble form of PrP, or to cell-associated PrP. Interestingly, in one study the effective peptide inhibitors tended to form β-sheet-rich structures by themselves (95). A slightly different approach has been used for identifying peptidyl inhibitors for use with huntingtin. Using phage display to screen a combinatorial peptide library, Nagai and coworkers identified several tryptophan-rich 11-mers with anti-aggregation activity against poly Q-containing proteins (96).

SUMMARY AND FUTURE DIRECTIONS

Promising advances have been made in designing compounds that specifically target self-assembling polypeptides and inhibit their adverse side effects in vitro. Bringing these compounds to the clinic requires not only development of combinatorial libraries and effective high-throughput screening methods, but also advances in our basic understanding of the conformational changes underlying conversion of monomer to aggregate, and the relationship between physicochemical properties and biological function.

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LITERATURE CITED

1. McGowan DP, van Roon-Mom W, Holloway H, Bates GP, Mangiarini L, et al. 2000. Amyloid-like inclusions in Huntington's disease. *Neuroscience* 100:677–80
2. Vassar R, Citron M. 2000. Abeta-generating enzymes: recent advances in beta- and gamma-secretase research. *Neuron* 27:419–22
3. Evert BO, Wullner U, Klockgether T. 2000. Cell death in polyglutamine diseases. *Cell Tissue Res.* 301:189–204
4. Mende-Mueller LM, Toneff T, Hwang SR, Chesselet MF, Hook VY. 2001. Tissue-specific proteolysis of Huntingtin (htt) in human brain: evidence of enhanced levels of N- and C-terminal htt

fragments in Huntington's disease striatum. *J. Neurosci.* 21:1830–37
5. Caughey B. 2000. Transmissible spongiform encephalopathies, amyloidoses and yeast prions: common threads? *Nat. Med.* 6:751–54
6. Kallberg Y, Gustafsson M, Persson B, Thyberg J, Johansson J. 2001. Prediction of amyloid fibril-forming proteins. *J. Biol. Chem.* 276:12945–50
7. Chiti F, Taddei N, Bucciantini M, White P, Ramponi G, Dobson CM. 2000. Mutational analysis of the propensity for amyloid formation by a globular protein. *EMBO J.* 19:1441–49
8. West MW, Wang W, Patterson J, Mancias JD, Beasley JR, Hecht MH. 1999. De novo amyloid proteins from designed combinatorial libraries. *Proc. Natl. Acad. Sci. USA* 96:11211–16
9. Barrow CJ, Yasuda A, Kenny PT, Zagorski MG. 1992. Solution conformations and aggregational properties of synthetic amyloid beta-peptides of Alzheimer's disease. Analysis of circular dichroism spectra. *J. Mol. Biol.* 225:1075–93
10. Talafous J, Marcinowski KJ, Klopman G, Zagorski MG. 1994. Solution structure of residues 1-28 of the amyloid beta-peptide. *Biochemistry* 33:7788–96
11. Kohno T, Kobayashi K, Maeda T, Sato K, Takashima A. 1996. Three-dimensional structures of the amyloid beta peptide (25-35) in membrane-mimicking environment. *Biochemistry* 35:16094–104
12. Fraser PE, Nguyen JT, Surewicz WK, Kirschner DA. 1991. pH-dependent structural transitions of Alzheimer amyloid peptides. *Biophys. J.* 60:1190–201
13. Fraser PE, McLachlan DR, Surewicz WK, Mizzen CA, Snow AD, et al. 1994. Conformation and fibrillogenesis of Alzheimer A beta peptides with selected substitution of charged residues. *J. Mol. Biol.* 244:64–73
14. Zhang Y, Swietnicki W, Zagorski MG, Surewicz WK, Sonnichsen FD. 2000. Solution structure of the E200K variant of human prion protein. Implications for the mechanism of pathogenesis in familial prion diseases. *J. Biol. Chem.* 275:33650–54
15. Massi F, Peng JW, Lee JP, Straub JE. 2001. Simulation study of the structure and dynamics of the Alzheimer's amyloid peptide congener in solution. *Biophys. J.* 80:31–44
16. Ragg E, Tagliavini F, Malesani P, Monticelli L, Bugiani O, et al. 1999. Determination of solution conformations of PrP106-126, a neurotoxic fragment of prion protein, by 1H NMR and restrained molecular dynamics. *Eur. J. Biochem.* 266: 1192–200
17. De Gioia L, Selvaggini C, Ghibaudi E, Diomede L, Bugiani O, et al. 1994. Conformational polymorphism of the amyloidogenic and neurotoxic peptide homologous to residues 106-126 of the prion protein. *J. Biol. Chem.* 269:7859–62
18. Nguyen J, Baldwin MA, Cohen FE, Prusiner SB. 1995. Prion protein peptides induce a-helix to b-sheet conformational transition. *Biochemistry* 34:4186–92
19. Zahn R, Liu A, Luhrs T, Riek R, von Schroetter C, et al. 2000. NMR solution structure of the human prion protein. *Proc. Natl. Acad. Sci. USA* 97:145–50
20. Thompson A, White AR, McLean C, Masters CL, Cappai R, Barrow CJ. 2000. Amyloidogenicity and neurotoxicity of peptides corresponding to the helical regions of PrP(C). *J. Neurosci. Res.* 62:293–301
21. Swietnicki W, Morillas M, Chen SG, Gambetti P, Surewicz WK. 2000. Aggregation and fibrillization of the recombinant human prion protein huPrP90–231. *Biochemistry* 39:424–31
22. Salmona M, Malesani P, De Gioia L, Gorla S, Bruschi M, et al. 1999. Molecular determinants of the physicochemical properties of a critical prion protein region comprising residues 106-126. *Biochem. J.* 342:207–14
23. Hosszu LL, Baxter NJ, Jackson GS,

Power A, Clarke AR, et al. 1999. Structural mobility of the human prion protein probed by backbone hydrogen exchange. *Nat. Struct. Biol.* 6:740–43

24. Maiti NR, Surewicz WK. 2001. The role of disulfide bridge in the folding and stability of the recombinant human prion protein. *J. Biol. Chem.* 276:2427–31
25. Jackson GS, Hosszu LL, Power A, Hill AF, Kenney J, et al. 1999. Reversible conversion of monomeric human prion protein between native and fibrilogenic conformations. *Science* 283:1935–37
26. Guilbert C, Ricard F, Smith JC. 2000. Dynamic simulation of the mouse prion protein. *Biopolymers* 54:406–15
27. Inouye H, Bond J, Baldwin MA, Ball HL, Prusiner SB, Kirschner DA. 2000. Structural changes in a hydrophobic domain of the prion protein induced by hydration and by Ala → Val and Pro → Leu substitutions. *J. Mol. Biol.* 300:1283–96
28. Perutz MF, Johnson T, Suzuki M, Finch JT. 1994. Glutamine repeats as polar zippers: their possible role in inherited neurodegenerative diseases. *Proc. Natl. Acad. Sci. USA* 91:5355–58
29. Monoi H, Futaki S, Kugimiya S, Minakata H, Yoshihara K. 2000. Poly-L-glutamine forms cation channels: relevance to the pathogenesis of the polyglutamine diseases. *Biophys. J.* 78:2892–99
30. Fraser PE, Duffy LK, O'Malley MB, Nguyen J, Inouye H, Kirschner DA. 1991. Morphology and antibody recognition of synthetic beta-amyloid peptides. *J. Neurosci. Res.* 28:474–85
31. Stine WB Jr, Snyder SW, Ladror US, Wade WS, Miller MF, et al. 1996. The nanometer-scale structure of amyloid-beta visualized by atomic force microscopy. *J. Protein Chem.* 15:193–203
32. Harper JD, Wong SS, Lieber CM, Lansbury PT Jr. 1999. Assembly of A beta amyloid protofibrils: an in vitro model for a possible early event in Alzheimer's disease. *Biochemistry* 38:8972–80
33. Blackley HK, Sanders GH, Davies MC, Roberts CJ, Tendler SJ, Wilkinson MJ. 2000. In-situ atomic force microscopy study of beta-amyloid fibrillization. *J. Mol. Biol.* 298:833–40
34. Malinchik SB, Inouye H, Szumowski KE, Kirschner DA. 1998. Structural analysis of Alzheimer's beta(1-40) amyloid: protofilament assembly of tubular fibrils. *Biophys. J.* 74:537–45A
35. Kheterpal I, Zhou S, Cook KD, Wetzel R. 2000. Abeta amyloid fibrils possess a core structure highly resistant to hydrogen exchange. *Proc. Natl. Acad. Sci. USA* 97:13597–601
36. Tjernberg LO, Callaway DJ, Tjernberg A, Hahne S, Lilliehook C, et al. 1999. A molecular model of Alzheimer amyloid beta-peptide fibril formation. *J. Biol. Chem.* 274:12619–25
37. Serpell LC, Blake CC, Fraser PE. 2000. Molecular structure of a fibrillar Alzheimer's A beta fragment. *Biochemistry* 39:13269–75
38. Benzinger TL, Gregory DM, Burkoth TS, Miller-Auer H, Lynn DG, et al. 2000. Two-dimensional structure of beta-amyloid(10-35) fibrils. *Biochemistry* 39: 3491–99
39. Antzutkin ON, Balbach JJ, Leapman RD, Rizzo NW, Reed J, Tycko R. 2000. Multiple quantum solid-state NMR indicates a parallel, not antiparallel, organization of beta-sheets in Alzheimer's beta-amyloid fibrils. *Proc. Natl. Acad. Sci. USA* 97:13045–50
40. Forloni G, Angeretti N, Chiesa R, Monzani E, Salmona M, et al. 1993. Neurotoxicity of a prion protein fragment. *Nature* 362:543–46
41. Baskakov IV, Legname G, Prusiner SB, Cohen FE. 2001. Folding of prion protein to its native alpha-helical conformation is under kinetic control. *J. Biol. Chem.* 276:19687–90
42. Jobling MF, Stewart LR, White AR, McLean C, Friedhuber A, et al. 1999. The hydrophobic core sequence modulates the

neurotoxic and secondary structure properties of the prion peptide 106–126. *J. Neurochem.* 73:1557–65

43. Scherzinger E, Sittler A, Schweiger K, Heiser V, Lurz R, et al. 1999. Self-assembly of polyglutamine-containing huntingtin fragments into amyloid-like fibrils: implications for Huntington's disease pathology. *Proc. Natl. Acad. Sci. USA* 96:4604–9
44. Jarrett JT, Berger EP, Lansbury PT Jr. 1993. The C-terminus of the beta protein is critical in amyloidogenesis. *Ann. NY Acad. Sci.* 695:144–48
45. Tomski SJ, Murphy RM. 1992. Kinetics of aggregation of synthetic beta-amyloid peptide. *Arch. Biochem. Biophys.* 294:630–38
46. Snyder SW, Ladror US, Wade WS, Wang GT, Barrett LW, et al. 1994. Amyloid-beta aggregation: selective inhibition of aggregation in mixtures of amyloid with different chain lengths. *Biophys. J.* 67:1216–28
47. Lomakin A, Teplow DB, Kirschner DA, Benedek GB. 1997. Kinetic theory of fibrillogenesis of amyloid beta-protein. *Proc. Natl. Acad. Sci. USA* 94:7942–47
48. Inouye H, Kirschner DA. 2000. A beta fibrillogenesis: kinetic parameters for fibril formation from congo red binding. *J. Struct. Biol.* 130:123–29
49. Pallitto MM, Murphy RM. 2001. A mathematical model of the kinetics of beta-amyloid fibril growth from the denatured state. *Biophys. J.* 81:1805–22
50. Post K, Pitschke M, Schafer O, Wille H, Appel TR, et al. 1998. Rapid acquisition of beta-sheet structure in the prion protein prior to multimer formation. *Biol. Chem.* 379:1307–17
51. Wildegger G, Liemann S, Glockshuber R. 1999. Extremely rapid folding of the C-terminal domain of the prion protein without kinetic intermediates. *Nat. Struct. Biol.* 6:550–53
52. Harrison PM, Chan HS, Prusiner SB, Cohen FE. 2001. Conformational propagation with prion-like characteristics in a simple model of protein folding. *Protein Sci.* 10:819–35
53. Tseng BP, Esler WP, Clish CB, Stimson ER, Ghilardi JR, et al. 1999. Deposition of monomeric, not oligomeric, Abeta mediates growth of Alzheimer's disease amyloid plaques in human brain preparations. *Biochemistry* 38:10424–31
54. Esler WP, Stimson ER, Jennings JM, Vinters HV, Ghilardi JR, et al. 2000. Alzheimer's disease amyloid propagation by a template-dependent dock-lock mechanism. *Biochemistry* 39:6288–95
55. Esler WP, Felix AM, Stimson ER, Lachenmann MJ, Ghilardi JR, et al. 2000. Activation barriers to structural transition determine deposition rates of Alzheimer's disease a beta amyloid. *J. Struct. Biol.* 130:174–83
56. Cohen FE, Prusiner SB. 1998. Pathologic conformations of prion proteins. *Annu. Rev. Biochem.* 67:793–819
57. Kazantsev A, Preisinger E, Dranovsky A, Goldgaber D, Housman D. 1999. Insoluble detergent-resistant aggregates form between pathological and nonpathological lengths of polyglutamine in mammalian cells. *Proc. Natl. Acad. Sci. USA* 96:11404–9
58. Stumpf MP, Krakauer DC. 2000. Mapping the parameters of prion-induced neuropathology. *Proc. Natl. Acad. Sci. USA* 97:10573–77
59. Kellershohn N, Laurent M. 1998. Species barrier in prion diseases: a kinetic interpretation based on the conformational adaptation of the prion protein. *Biochem. J.* 334:539–45
60. Pike CJ, Burdick D, Walencewicz AJ, Glabe CG, Cotman CW. 1993. Neurodegeneration induced by beta-amyloid peptides in vitro: the role of peptide assembly state. *J. Neurosci.* 13:1676–87
61. Shearman MS, Ragan CI, Iversen LL. 1994. Inhibition of PC12 cell redox activity is a specific, early indicator of the mechanism of beta-amyloid-mediated

cell death. *Proc. Natl. Acad. Sci. USA* 91: 1470–74

62. Seilheimer B, Bohrmann B, Bondolfi L, Muller F, Stuber D, Dobeli H. 1997. The toxicity of the Alzheimer's beta-amyloid peptide correlates with a distinct fiber morphology. *J. Struct. Biol.* 119:59–71
63. Roher AE, Chaney MO, Kuo YM, Webster SD, Stine WB, et al. 1996. Morphology and toxicity of Abeta-(1-42) dimer derived from neuritic and vascular amyloid deposits of Alzheimer's disease. *J. Biol. Chem.* 271:20631–35
64. Hartley DM, Walsh DM, Ye CP, Diehl T, Vasquez S, et al. 1999. Protofibrillar intermediates of amyloid beta-protein induce acute electrophysiological changes and progressive neurotoxicity in cortical neurons. *J. Neurosci.* 19:8876–84
65. Ward RV, Jennings KH, Jepras R, Neville W, Owen DE, et al. 2000. Fractionation and characterization of oligomeric, protofibrillar and fibrillar forms of beta-amyloid peptide. *Biochem. J.* 348(Pt 1): 137–44
66. Ettaiche M, Pichot R, Vincent JP, Chabry J. 2000. In vivo cytotoxicity of the prion protein fragment 106-126. *J. Biol. Chem.* 275:36487–90
67. Brown DR. 2000. Prion protein peptides: optimal toxicity and peptide blockade of toxicity. *Mol. Cell Neurosci.* 15:66–78
68. Wille H, Prusiner SB, Cohen FE. 2000. Scrapie infectivity is independent of amyloid staining properties of the N-terminally truncated prion protein. *J. Struct. Biol.* 130:323–38
69. Li H, Li SH, Johnston H, Shelbourne PF, Li XJ. 2000. Amino-terminal fragments of mutant huntingtin show selective accumulation in striatal neurons and synaptic toxicity. *Nat. Genet.* 25:385–89
70. Kuemmerle S, Gutekunst CA, Klein AM, Li XJ, Li SH, et al. 1999. Huntington aggregates may not predict neuronal death in Huntington's disease. *Ann. Neurol.* 46: 842–49
71. Wellington CL, Singaraja R, Ellerby L, Savill J, Roy S, et al. 2000. Inhibiting caspase cleavage of huntingtin reduces toxicity and aggregate formation in neuronal and nonneuronal cells. *J. Biol. Chem.* 275:19831–38
72. Bence NF, Sampat RM, Kopito RR. 2001. Impairment of the ubiquitin-proteasome system by protein aggregation. *Science* 292:1552–55
73. Warrick JM, Chan HY, Gray-Board GL, Chai Y, Paulson HL, Bonini NM. 1999. Suppression of polyglutamine-mediated neurodegeneration in Drosophila by the molecular chaperone HSP70. *Nat. Genet.* 23:425–28
74. Carmichael J, Chatellier J, Woolfson A, Milstein C, Fersht AR, Rubinsztein DC. 2000. Bacterial and yeast chaperones reduce both aggregate formation and cell death in mammalian cell models of Huntington's disease. *Proc. Natl. Acad. Sci. USA* 97:9701–5
75. Ferrigno P, Silver PA. 2000. Polyglutamine expansions: proteolysis, chaperones, and the dangers of promiscuity. *Neuron* 26:9–12
76. Kisilevsky R, Lemieux LJ, Fraser PE, Kong X, Hultin PG, Szarek WA. 1995. Arresting amyloidosis in vivo using small-molecule anionic sulphonates or sulphates: implications for Alzheimer's disease. *Nat. Med.* 1:143–48
77. Lorenzo A, Yankner BA. 1994. Beta-amyloid neurotoxicity requires fibril formation and is inhibited by congo red. *Proc. Natl. Acad. Sci. USA* 91:12243–47
78. Heiser V, Scherzinger E, Boeddrich A, Nordhoff E, Lurz R, et al. 2000. Inhibition of huntingtin fibrillogenesis by specific antibodies and small molecules: implications for Huntington's disease therapy. *Proc. Natl. Acad. Sci. USA* 97:6739–44
79. Klunk WE, Debnath ML, Koros AM, Pettegrew JW. 1998. Chrysamine-G, a lipophilic analogue of Congo red, inhibits A beta-induced toxicity in PC12 cells. *Life Sci.* 63:1807–14

80. Howlett DR, Perry AE, Godfrey F, Swatton JE, Jennings KH, et al. 1999. Inhibition of fibril formation in beta-amyloid peptide by a novel series of benzofurans. *Biochem. J.* 340:283–89
81. Tomiyama T, Shoji A, Kataoka K, Suwa Y, Asano S, et al. 1996. Inhibition of amyloid beta protein aggregation and neurotoxicity by rifampicin. Its possible function as a hydroxyl radical scavenger. *J. Biol. Chem.* 271:6839–44
82. Caughey WS, Raymond LD, Horiuchi M, Caughey B. 1998. Inhibition of protease-resistant prion protein formation by porphyrins and phthalocyanines. *Proc. Natl. Acad. Sci. USA* 95:12117–22
83. Reixach N, Crooks E, Ostresh JM, Houghten RA, Blondelle SE. 2000. Inhibition of beta-amyloid-induced neurotoxicity by imidazopyridoindoles derived from a synthetic combinatorial library. *J. Struct. Biol.* 130:247–58
84. Kuner P, Bohrmann B, Tjernberg LO, Naslund J, Huber G, et al. 2000. Controlling polymerization of beta-amyloid and prion-derived peptides with synthetic small molecule ligands. *J. Biol. Chem.* 275:1673–78
85. Lecerf JM, Shirley TL, Zhu Q, Kazantsev A, Amersdorfer P, et al. 2001. Human single-chain Fv intrabodies counteract in situ huntingtin aggregation in cellular models of Huntington's disease. *Proc. Natl. Acad. Sci. USA* 98:4764–69
86. Solomon B, Koppel R, Frankel D, Hanan-Aharon E. 1997. Disaggregation of Alzheimer beta-amyloid by site-directed mAb. *Proc. Natl. Acad. Sci. USA* 94:4109–12
87. Tjernberg LO, Naslund J, Lindqvist F, Johansson J, Karlstrom AR, et al. 1996. Arrest of beta-amyloid fibril formation by a pentapeptide ligand. *J. Biol. Chem.* 271:8545–48
88. Soto C, Sigurdsson EM, Morelli L, Kumar RA, Castano EM, Frangione B. 1998. Beta-sheet breaker peptides inhibit fibrillogenesis in a rat brain model of amyloidosis: implications for Alzheimer's therapy. *Nat. Med.* 4:822–26
89. Hughes E, Burke RM, Doig AJ. 2000. Inhibition of toxicity in the beta-amyloid peptide fragment beta-(25-35) using N-methylated derivatives: a general strategy to prevent amyloid formation. *J. Biol. Chem.* 275:25109–15
90. Ghanta J, Shen CL, Kiessling LL, Murphy RM. 1996. A strategy for designing inhibitors of beta-amyloid toxicity. *J. Biol. Chem.* 271:29525–28
91. Findeis MA, Musso GM, Arico-Muendel CC, Benjamin HW, Hundal AM, et al. 1999. Modified-peptide inhibitors of amyloid beta-peptide polymerization. *Biochemistry* 38:6791–800
92. Pallitto MM, Ghanta J, Heinzelman P, Kiessling LL, Murphy RM. 1999. Recognition sequence design for peptidyl modulators of beta-amyloid aggregation and toxicity. *Biochemistry* 38:3570–78
93. Lowe TL, Strzelec A, Kiessling LL, Murphy RM. 2001. Structure-function relationships for inhibitors of beta-amyloid toxicity containing the recognition sequence KLVFF. *Biochemistry* 40:7882–89
94. Chabry J, Priola SA, Wehrly K, Nishio J, Hope J, Chesebro B. 1999. Species-independent inhibition of abnormal prion protein (PrP) formation by a peptide containing a conserved PrP sequence. *J. Virol.* 73:6245–50
95. Horiuchi M, Baron GS, Xiong LW, Caughey B. 2001. Inhibition of interactions and interconversions of prion protein isoforms by peptide fragments from the C-terminal folded domain. *J. Biol. Chem.* 276:15489–97
96. Nagai Y, Tucker T, Ren H, Kenan DJ, Henderson BS, et al. 2000. Inhibition of polyglutamine protein aggregation and cell death by novel peptides identified by phage display screening. *J. Biol. Chem.* 275:10437–42

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