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Review

Paradigm Shifts in Alzheimer's Disease and Other Neurodegenerative Disorders: The Emerging Role of Oligomeric Assemblies

Marina D. Kirkitadze,[1,2] **Gal Bitan,**[1,2] **and David B. Teplow**[1,2]*
[1]Department of Neurology, Harvard Medical School, Boston, Massachusetts
[2]Center for Neurologic Diseases, Brigham and Women's Hospital, Boston, Massachusetts

Alzheimer's disease (AD) is a progressive, neurodegenerative disorder characterized by amyloid deposition in the cerebral neuropil and vasculature. These amyloid deposits comprise predominantly fragments and full-length (40 or 42 residue) forms of the amyloid β-protein (Aβ) organized into fibrillar assemblies. Compelling evidence indicates that factors that increase overall Aβ production or the ratio of longer to shorter forms, or which facilitate deposition or inhibit elimination of amyloid deposits, cause AD or are risk factors for the disease. In vitro studies have demonstrated that fibrillar Aβ has potent neurotoxic effects on cultured neurons. In vivo experiments in non-human primates have demonstrated that Aβ fibrils directly cause pathologic changes, including tau hyperphosphorylation. In concert with histologic studies revealing a lack of tissue injury in areas of the neuropil in which non-fibrillar deposits were found, these data suggested that fibril assembly was a prerequisite for Aβ-mediated neurotoxicity in vivo. Recently, however, both in vitro and in vivo studies have revealed that soluble, oligomeric forms of Aβ also have potent neurotoxic activities, and in fact, may be the proximate effectors of the neuronal injury and death occurring in AD. A paradigm shift is thus emerging that necessitates the reevaluation of the relative importance of polymeric (fibrillar) vs. oligomeric assemblies in the pathobiology of AD. In addition to AD, an increasing number of neurodegenerative disorders, including Parkinson's disease, familial British dementia, familial amyloid polyneuropathy, amyotrophic lateral sclerosis, and prion diseases, are associated with abnormal protein assembly processes. The archetypal features of the assembly-dependent neuropathogenetic effects of Aβ may thus be of relevance not only to AD but to these other disorders as well.

Key words: amyloid β-protein, neurotoxicity, neurodegeneration, protofibrils, ADDLs, oligomers, protein folding, protein assembly

In 1906, Alois Alzheimer reported the histopathologic analysis of an unusual case of dementia (Alzheimer, 1906). The "peculiar substance" that Alzheimer observed (Alzheimer, 1907) in the cerebral cortex of this patient, August D., was later found to be composed of fibrils of the amyloid β-protein (Aβ) (Teplow, 1998; Selkoe, 2001). Not surprisingly, an important paradigm guiding efforts over the last century toward therapeutic intervention in AD has been that fibril formation by Aβ leads to neurodegeneration and death (Hardy and Higgins, 1992). This paradigm has provided the theoretical basis for recent therapeutic approaches demonstrating promise for amyloid elimination (Schenk et al., 2000; Cherny et al., 2001; DeMattos et al., 2001). Mounting evidence, however, suggests that soluble, oligomeric Aβ assemblies cause substantial neuronal dysfunction before the appearance of amyloid deposits (Klein et al., 2001). Amyloid fibril formation and deposition thus may be end stages of a process in which the key pathogenetic events occur early and are mediated by oligomeric assemblies. If so, fibril elimination strategies may prove ineffective or counterproductive (Biospace.com, 2002; Pasinetti et al., 2002). Space limitations prevent a full reconciliation of the new findings about soluble oligomers with the extensive body of experimental work linking fibrils to neurodegeneration. It should be noted, however, that the two types of assembly exist in equilibrium. This relationship means that kinetic, thermodynamic, and physiologic factors affecting the formation, metabolism, and activity of one structure also can affect the other. In this review, we restrict our attention to

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*Correspondence to: David B. Teplow, PhD, Center for Neurologic Diseases, Brigham and Women's Hospital, 77 Avenue Louis Pasteur (HIM 756), Boston, MA 02115-5727. E-mail: teplow@cnd.bwh.harvard.edu

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recent in vivo and in vitro studies of Aβ oligomerization and its role in neuronal dysfunction. In addition, we survey work on other proteins and peptides for which an etiologic association between abnormal oligomerization and neurodegeneration has been suggested. Taken together, evidence accumulated in these studies supports a refocusing of research efforts away from fibril formation per se and toward the examination of the initial stages of protein folding and oligomerization.

Aβ ASSEMBLY AND NEURODEGENERATION

By definition, Alzheimer's disease is characterized by the formation of neuritic plaques and neurofibrillary tangles (NFT) (Khachaturian, 1985; Mirra, 1991; Esiri, 2001). The neuronal injury and death associated with this extracellular amyloid deposition and intracellular NFT accumulation, and with amyloid angiopathy, likely contribute to the clinical features of AD. The relative roles of each of these phenomena in the pathogenesis of AD, however, has been contentious. For this reason, we preface this section of the review with a brief discussion of the roles of Aβ and tau in AD. The hypothesis that amyloid plaque formation is the proximate cause of AD has received the greatest attention and is supported by the largest body of evidence (Hardy and Higgins, 1992; Hardy, 1997). Significant experimental support, however, also exists for the involvement of the microtubule-associated protein tau (Mandelkow and Mandelkow, 1998). Of relevance to the question of the roles of Aβ and tau in AD neuropathology is the finding that mouse double transgenics, expressing both the human amyloid β-protein precursor (AβPP) and tau, exhibit enhanced NFT formation relative to the single, tau transgenic control animals (Lewis et al., 2001). Formation of NFT also has been induced by direct injection of Aβ(1–42) into the brains of transgenic mice expressing human tau (Gotz et al., 2001), although tangle formation in neurons does not necessarily require their proximity to amyloid deposits (Lewis et al., 2001). Masliah et al. (2001) studied transgenic mice expressing the V717F form of AβPP and found that amyloid formation preceded the appearance of aggregates containing phosphorylated tau epitopes associated with AD. These studies suggest that (over)expression of AβPP or elevation of Aβ concentration induce tau assembly. Conversely, tau itself may mediate Aβ-induced neuronal degeneration. In a cell culture model of fibrillar Aβ-induced neuronal injury, hippocampal neurons isolated from transgenic mice expressing either mouse or human tau degenerated, whereas cells from tau knockout animals remained viable (Rapoport et al., 2002). It is also possible, however, that neither Aβ nor tau play the *key* role in AD pathogenesis. For example, Mesulam (1999) has proposed an intriguing and reasonable scenario in which a variety of factors first compromise the neuroplastic potential of the brain, after which Aβ and tau both become involved in the neurodegenerative process (Terry, 2001). To advance our understanding of the etiology of AD, it is critical to test each hypothesis in as critical and unbiased a manner as possible. We focus on evidence relating to the role of Aβ assembly in neurodegeneration. Our goal is to provide perspective in this area to facilitate the design and execution of experiments able to address the most critical underpinnings of the "amyloid cascade hypothesis" and reveal key pathogenetic mechanisms in AD.

Human Studies

Past efforts to correlate amyloid deposition with measures of clinical status have been problematic (Selkoe, 1994). Simple measures of total amyloid burden or plaque number do not correlate convincingly with disease severity. Greater promise was shown with region-specific quantitation of plaque burden. For example, Cummings et al. (1996) reported that the area of entorhinal cortex occupied by Aβ correlated significantly with global cognitive impairment. Näslund et al. (2000) showed that levels of Aβ peptides ending at either Val^{40} or Ala^{42} were elevated in AD and directly correlated with dementia, as measured by CDR scores (Hughes, 1982; Morris, 1993). They also reported that increases in Aβ levels in the frontal cortex preceded the appearance of NFT, suggesting an important role for Aβ early in AD (Näslund et al., 2000). Wang et al. (1999) showed that the progression from normal aging to "pathologic" aging, and then on to frank AD, is accompanied by a consistent, statistically significant increase in insoluble Aβ. Interestingly, significant increases in soluble Aβ also correlated with the severity of brain pathology. Subsequent studies have confirmed and extended this latter result. McLean et al. (1999) used Western blotting to quantify levels of soluble and insoluble Aβ. They observed a three-fold increase in soluble Aβ in histologically-confirmed AD cases relative to control cases. Of particular interest was the finding that levels of soluble Aβ directly correlated with NFT density. In contrast, the level of insoluble Aβ (also a measure of total amyloid load) only discriminated AD cases from controls, but did not correlate with other disease measures. Lue et al. (1999) measured 11 different parameters of synapse, Aβ, plaque, and NFT level in AD patients, normal patients, and patients with pronounced neurohistopathology in the absence of clinical signs of AD. The most significant correlate of synapse loss was the level of Aβ(1–40), whether present in soluble or insoluble fractions. Levels of soluble Aβ(1–40) were the most significant discriminator among AD patients, "high pathology" controls, and normal individuals. Pitschke et al. (1998), using spectroscopic techniques, have demonstrated an association of soluble CSF Aβ with AD. In Down's syndrome, patients surviving into their fourth decade invariably display histopathologic evidence of AD (Rumble et al., 1989). This is thought to be a gene dosage effect resulting from the trisomy of chromosome 21 (Rumble et al., 1989; Prasher et al., 1998), on which the AβPP gene resides (Blacker and Tanzi, 1998). Teller et al. (1996) have measured levels of soluble Aβ(1–42) in the brains of Down's patients over a 6-decade age range, including pre partum. They were able to detect soluble Aβ(1–42) in these patients when no peptide was detectable in normal controls (Teller et al.,

1996). Taken together, the studies discussed above suggest that one hypothesis worthy of testing is that soluble Aβ species are critical early contributors to the pathogenesis of AD.

Studies of Transgenic Animals

A number of excellent animal models of AD are available that recapitulate specific aspects of the disease process. In transgenic mice overexpressing human AβPP or presenilins, early and extensive amyloid deposition is observed (Duff and Rao, 2001). Initial studies of transgenic animals demonstrated a correlation between amyloid deposition and neuronal dysfunction (Games et al., 1995; Hsiao et al., 1996; Masliah et al., 1996). Subsequently, monitoring of a variety of neurophysiological measures revealed that significant neuronal injury occurred before the appearance of plaques (Dodart et al., 1999; Hsia et al., 1999; Larson et al., 1999; Moechars et al., 1999; Kumar-Singh et al., 2000; Mucke et al., 2000). A study of specific spatial learning deficits in AβPP-overexpressing mice (Koistinaho et al., 2001) suggested that memory impairment might be caused by diffuse Aβ deposits, but not plaques. Chen et al. (2000) demonstrated that subneurotoxic concentrations of Aβ could strongly suppress long-term synaptic plasticity in the hippocampus. This effect may underlie the memory deficits occurring in Alzheimer's disease before neuronal cell loss. Careful quantitative studies in transgenic mice expressing FAD-associated human presenilin (PS) genes have shown that neurodegeneration was significantly accelerated in aged (older than 13 months) mice, in the absence of amyloid plaque formation (Chui et al., 1999). Walsh et al. (2002) have provided recently additional evidence that Aβ oligomers are potent neurotoxins. In studies in normal rats, oligomeric Aβ assemblies injected intracerebrally caused significant inhibition of hippocampal long-term potentiation, whereas monomeric Aβ had no effect. Thus, in mice and rats, as in humans, neuronal dysfunction caused by soluble, oligomeric Aβ assemblies may occur independently of, and before, amyloid deposition. Direct experimental support for the primacy of oligomer-mediated neuronal injury has come from very recent studies demonstrating that both subchronic (6-week) and acute (single) passive immunization of AβPP-transgenic mice with Aβ-specific antibodies can rapidly reverse memory impairment without affecting total amyloid burden (Dodart et al., 2002).

In Vitro Studies

The association of amyloid deposition with the progression of AD has stimulated studies examining the potential correlation of Aβ assembly state and neurotoxicity. Early work in this area demonstrated that Aβ fibrils were neurotoxic (Pike et al., 1991, 1993; Roher, 1991). This observation has been confirmed many times (Kuroda and Kawahara, 1994; Howlett et al., 1995; Forloni et al., 1996; Weldon et al., 1998), a fact lending considerable importance to efforts to elucidate the structural and thermodynamic features of the fibril assembly process (for reviews, see Teplow, 1998; Serpell, 2000). In 1997, two groups reported the discovery of a fibril assembly intermediate, the protofibril (Harper et al., 1997; Walsh et al., 1997). This structure appears to be the immediate precursor of amyloid-type fibrils and is typically observed as a short, flexible, filamentous assembly (Harper et al., 1999; Walsh et al., 1999). Protofibrils have diameters of ~5 nm and often display a beaded appearance (Nybo et al., 1999; Walsh et al., 1999; Blackley et al., 2000).

One goal of fibril-centric therapeutic strategies is the dissociation and elimination of fibrils in situ in affected areas of the brain. If dissociation were to produce protofibrils, and these assemblies were themselves toxic, however, then the strategy would fail. For this reason, studies have been done to determine if protofibrils were neurotoxic. Using three different approaches, lactate dehydrogenase (LDH) release, dye (MTT) metabolism, and electrical activity, protofibrils were found to be potent neurotoxins (Hartley et al., 1999; Walsh et al., 1999). Protofibrils added to cultured primary rat cortical neurons caused LDH release (a measure of cell death) in a concentration- and time-dependent manner. However, because cell death assays can require extended incubation times (~1 week), associating toxic activity with a particular assembly can be problematic. MTT assays, which reveal physiologic effects after incubation times of a few hours (Shearman et al., 1994, 1995; Howlett et al., 1995; Liu and Schubert, 1997), were thus used to determine whether protofibrils could affect the physiology of cultured rat neurons over a time-scale during which little or no protofibril→fibril conversion occurred. Significant inhibition of MTT metabolism was observed and the effects were proportionate to protofibril concentration (Walsh et al., 1999). Finally, when protofibrils or fibrils were added to neurons and electrical activity monitored, a rapid, sustained increase in excitatory post-synaptic currents (EPSCs) was recorded (Hartley et al., 1999). Highly significant increases in the frequency of action potentials and the magnitude and frequency of membrane depolarizations also were observed. These effects may underlie certain of the toxic consequences of Aβ assemblies in vivo.

Support for the involvement of protofibrils in neurodegeneration recently has come from an intriguing study of a kindred in northern Sweden in which early onset AD is caused by an E693G mutation in the AβPP gene (Nilsberth et al., 2001). Carriers of this "Arctic" mutation show decreased plasma levels of Aβ(1–40) and Aβ(1–42), contrary to the effects of other AβPP mutations. Kinetics studies, however, showed that the Arctic form of Aβ(1–40) formed protofibrils at a higher rate and in greater quantities than did the wild-type peptide, suggesting that the pathogenetic mechanism of the Arctic form of AD involves protofibrils. Taken together with the fact that protofibrils are toxic to neurons in vitro, these data emphasize the potential importance of protofibrils in AD pathogenesis and the necessity to better understand protofibril formation. In fact, it has been postulated that

protofibril-induced neuronal injury may be a universal and central feature in neurodegenerative disorders (Haass and Steiner, 2001).

In addition to protofibrils, Aβ(1–42) forms globular assemblies termed ADDLs (Aβ-derived diffusible ligands) (Oda et al., 1995; Lambert et al., 1998). The precise structural relationship between ADDLs and protofibrils remains to be determined. Like protofibrils, however, ADDLs are potent neurotoxins. ADDLs can kill mature neurons in organotypic CNS cultures at nanomolar concentrations (Lambert et al., 1998). Neuronal dysfunction caused by ADDLs occurs before cellular degeneration (Lambert et al., 1998). For example, ADDLs inhibit hippocampal long-term potentiation (Lambert et al., 1998; Wang et al., 2002), indicating an immediate impact on signal transduction. Functional studies suggest that this effect may involve the Fyn kinase (Lambert et al., 1998). Recent toxicity studies using neuroblastoma N2A cells have suggested that ADDLs are more toxic than fibrillar forms of Aβ(1–42) (Manelli et al., 2001). Interestingly, the concentration of ADDL-like oligomers in soluble extracts of AD brain has been found to be elevated relative to that in normal controls (Gong et al., 2001). The unique ability of Aβ(1–42) [relative to Aβ(1–40)] to form ADDLs offers one explanation for the strong clinical association of Aβ(1–42) with AD.

Aβ Assembly and Neurodegeneration: Conclusions

The multifactorial etiology of AD complicates the construction of simple schemes of disease pathogenesis. Late in the disease process, anatomic and physiologic changes in the brain are abundant and obvious. This has led to the correlation of a variety of markers with AD (Percy et al., 2000). For most, if not all of these markers, their causative role in AD remains at issue. Ideally, one would like to identify factors that *initiate* the AD pathogenetic cascade. This would provide opportunities to intervene at early stages of the disease, before irrevocable neuronal injury and loss. In the case of Aβ, the experimental results discussed above support, but do not prove formally, the hypothesis that soluble, pre-fibrillar assemblies are early and powerful effectors of neuropathogenesis. Thoughtful strategies for combined in vivo and in vitro study of the assembly and biological activities of Aβ assemblies are necessary to test further this intriguing idea. The laws of physics, which control the folding, assembly, and physiologic interactions of Aβ, operate in all milieus. Therefore, basic mechanistic insights into Aβ assembly achieved through biophysical studies in vitro are of great relevance. Aβ metabolism in vivo, however, involves more than simple protein expression and homotypic interaction. A full understanding of Aβ biology requires that the activities of heterotypic (non-Aβ) factors (e.g., chaperones, membrane components) in modulating Aβ assembly and activity be integrated with the knowledge obtained from in vitro studies.

ABERRANT PROTEIN ASSEMBLY AND OTHER NEURODEGENERATIVE DISORDERS

AD is the most prevalent late-onset neurodegenerative disorder and the most common cause of dementia (Selkoe, 1991). For this reason, it is particularly intensely studied. The attention devoted to AD has produced a wealth of information about the biology of Aβ and its role in the disease. Importantly, features of Aβ assembly are shared among other proteins associated with neurodegenerative disorders, making Aβ assembly an archetypal process. We discuss below examples of assembly-dependent neurodegenerative diseases in which abnormal protein or peptide assembly may play a causative role. In each of these cases, amyloid-type fibrils are formed in an assembly process that also can involve oligomeric and protofibrillar intermediates.

Parkinson's Disease

Parkinson's disease (PD) is characterized by extensive loss of dopaminergic neurons in the substantia nigra (Lewy, 1912). The histopathologic hallmark of PD is the Lewy body, a dense, intracytoplasmic, protein aggregate (Lewy, 1912; Tretiakoff, 1919). α-Synuclein, a small protein expressed at high levels in brain tissue and localized at presynaptic terminals, has been identified as a major component of Lewy bodies (Baba et al., 1998; Spillantini et al., 1998). In a small number of cases, early-onset PD is caused by mutations in the α-synuclein gene. These mutations result in the amino acid substitutions A30P (Kruger et al., 1998) or A53T (Polymeropoulos et al., 1997). The linkage of these substitutions with PD suggested that formation of Lewy bodies and subsequent neuronal degeneration might be related to changes in the biophysical behavior of wild-type and mutant proteins. To address this question, wild-type and both mutant forms of α-synuclein have been studied in vitro (Conway et al., 1998; Narhi et al., 1999; Wood et al., 1999). α-Synuclein is "natively disordered" in solution (Conway et al., 1998) but both of the mutant proteins can form fibrils and discrete spherical assemblies after prolonged incubation (Conway et al., 1998; Narhi et al., 1999). Subsequent work examined the rates of disappearance of monomeric α-synuclein and the appearance of fibrils (Conway et al., 2000). The A53T protein, or an equimolar mixture of A53T and wild-type proteins, fibrillized more rapidly than did wild-type α-synuclein alone. In contrast, the A30P protein alone, or the corresponding equimolar mixture of A30P and wild-type proteins, both fibrillized more slowly than did wild-type protein. The difference between these trends suggested the existence of non-fibrillar α-synuclein oligomers, some of which were separated from fibrillar and monomeric α-synuclein by sedimentation followed by gel filtration chromatography. Atomic force microscopy was used to study the morphology of the α-synuclein assemblies (Volles et al., 2001). A number of structures were observed, including spheres, protofibrils, and rings. To evaluate whether these assemblies might be membrane active, Volles et al. mixed mo-

nomeric, protofibrillar, and fibrillar forms of α-synuclein with synthetic vesicles. They found that protofibrillar wild-type, A30P, and A53T α-synuclein, in contrast to monomeric and fibrillar forms of the peptides, could bind to and permeabilize these vesicles, suggesting a potential pathogenetic mechanism for PD (Volles et al., 2001). Consistent with this idea, α-synuclein has recently been shown to have biochemical properties and a structural motif similar to those of fatty acid-binding proteins (Sharon et al., 2001). In addition, both monomeric and higher molecular weight forms of α-synuclein isolated from cells or brain have been found to be associated with lipids (Sharon et al., 2001). Thus, as with Aβ, evidence suggests that α-synuclein may form oligomeric intermediates and that potent toxic activities of these structures may result from their interaction with cellular membranes. It is noteworthy that transgenic animals expressing both human AβPP and α-synuclein exhibit neuronal dysfunction earlier than do wild-type or α-synuclein single transgenic animals (Masliah et al., 2002). This phenotype appears to result from Aβ-dependent facilitation of α-synuclein assembly and accumulation.

Familial British Dementia

Familial British dementia (FBD) is an autosomal dominant, neurodegenerative disorder characterized by progressive spastic quadriparesis, cerebellar ataxia, and dementia (Worster-Drought et al., 1933; Griffiths et al., 1982). The neuropathology of FBD shares a number of features with AD, in particular the occurrence of parenchymal plaques, NFT, and vascular deposits (Ghiso et al., 2001). The amyloid angiopathy in FBD tends to be more severe and widespread than in AD (Ghiso et al., 2001). Immunohistochemical and biochemical analysis of plaques and vascular amyloid of FBD brains revealed that a 4 kDa peptide, ABri, is a primary component (Vidal et al., 1999). ABri is derived through proteolytic processing of a larger precursor encoded by the mutant BRI gene located on chromosome 13 (Vidal et al., 1999). The FBD mutation in BRI creates a Stop-to-Arg codon change resulting in an 11 amino acid C-terminal extension of the BRI protein (Vidal et al., 1999). ABri comprises the 34 C-terminal residues of the mutant BRI protein. The sequence and topography of the precursor protein are consistent with the existence of an intramolecular disulphide bond in the ABri peptide (El-Agnaf et al., 2001b). Cyclized (oxidized) ABri has, in fact, been shown to form Congo Red-positive fibrils in vitro (El-Agnaf, 2000). The fibril assembly process involves formation of soluble oligomers, protofibrils, and then amyloid-type fibrils (El-Agnaf et al., 2001a; Kim et al., 1999). These assemblies were not formed by wild-type peptide. Studies of the biological activity of ABri in cultures of human dopaminergic SHSY-5Y cells showed that the peptide caused alterations in the metabolism of MTT and induced LDH release (El-Agnaf et al., 2001a). Staining of the treated cells by Annexin V was consistent with the occurrence of an apoptotic process (El-Agnaf et al., 2001a). A finding of particular relevance was that non-fibrillar oligomeric species were more toxic than were protofibrils or mature fibrils (El-Agnaf et al., 2001a). Thus, although ABri and Aβ are non-homologous and dissimilar in primary structure, key features of their neuropathology, fibril assembly pathways, and oligomer neurotoxic activity are shared.

Familial Danish Dementia

Familiar Danish dementia (FDD) is an autosomal dominant disorder characterized by cataracts, deafness, progressive ataxia, and dementia (Strömgren, 1981; Strömgren et al., 1970). Similar to AD, neuropathological findings include cerebral amyloid angiopathy, hippocampal plaques, and neurofibrillary tangles (Strömgren, 1981). Isolation and amino acid sequence analysis of leptomeningeal fibrils has shown them to be composed of a peptide, ADan. Interestingly, ADan and ABri are encoded by the same ancestral BRI gene, but each results from a different mutation in this gene (Vidal et al., 2000). Like ABri, ADan comprises 34 amino acids at the C-terminus of the mutant BRI protein. The sequence of the C-terminal 12 amino acids of ADan, however, differs from that of ABri. This difference results from the fact that the FDD mutation, a 10-nucleotide duplication between codons 265 and 266 of the BRI gene, one codon before the normal stop codon 267 (Vidal et al., 2000), is distinct from the point mutation of FBD. An important structural feature is maintained between the two peptides, the presence of Cys^{5} and Cys^{22}, shown to be disulfide linked in ABri. The potential for identical disulfide bonding in ADan, and its N-terminal 22 amino acid identity with ABri, suggest that the two peptides could assemble in similar ways and thus produce similar oligomeric and protofibrillar species. One feature of the Danish disease that has been noted previously is the lack of Congo red staining in the hippocampus (Vidal et al., 2000), suggesting that non-fibrillar forms of ADan may effect the hippocampal neuronal dysfunction observed in these patients. Further study of ADan assembly and its biological effects are required to address these issues.

Familial Amyloid Polyneuropathy

In familial amyloid polyneuropathy (FAP) (Andrade, 1952), amyloid is deposited diffusely in the peripheral nervous system in nerve trunks, plexuses, and sensory and autonomic ganglia (Coimbra and Andrade, 1971a,b). Fibril accumulation is closely linked to neuronal degeneration (Said et al., 1984). In severely affected nerves, endoneurial contents are replaced by amyloid and few nerve fibers retain viability (Coimbra and Andrade, 1971b). The major component of FAP amyloid fibrils is transthyretin (TTR) (Costa et al., 1978). TTR is an abundant, homotetrameric plasma protein (Monaco, 2000). It is associated with retinol-binding protein and is the primary plasma carrier of L-thyroxine (Monaco, 2000). In addition to involvement in FAP, TTR also is linked to senile systemic amyloidosis (Westermark et al., 1990). Over 60 amyloidogenic TTR mutations have been identified (Damas and Saraiva, 2000; Hamilton, 2001). TTR has been crystallized and its structure solved (Blake, 1978; Hamilton et al., 1993), allowing detailed analysis of the

effects of disease-associated amino acid substitutions on its folding and assembly (Damas and Saraiva, 2000). A key finding is that destabilization of the TTR tetramer, either through mutation or alterations in environmental conditions (e.g., pH), can lead to tetramer dissociation and the production of a monomer having an alternatively-folded, amyloidogenic tertiary structure. Self-assembly of these altered monomers leads to the formation of oligomers, protofibrils (Lashuel et al., 1998, 1999), and fibrils (Lashuel et al., 1998). Recent studies by Sousa et al. (2001) have provided evidence that pre-fibrillar structures may produce neuronal stress in FAP patients early in the disease process. They showed that in peripheral nerves, non-fibrillar, Congo red-negative, TTR aggregates can be observed during the initial stage of the disease, before the observation of fibrils. Indirect evidence for a cytotoxic activity of the prefibrillar TTR structures was provided by immunocytochemical assessment of macrophage colony-stimulating factor (MCSF) levels, which showed early and continued expression at sites of TTR deposition. In vitro studies of TTR activity showed that caspase-3 activation could be induced by prefibrillar aggregates, suggesting that apoptotic cytotoxic mechanisms may operate in FAP. Thus, in FAP, as in AD, PD, and FBD, prefibrillar assemblies form and may play an important role in the neuropathogenetic process.

Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is an age-associated disease in which selective destruction of motor neurons occurs in the spinal cord, brain stem, and motor cortex (Brown, 1995). The progressive dysfunction of upper and lower motor neurons causes death from respiratory paralysis, usually within 5 years (Brownell et al., 1970; Brown, 1995; Cleveland, 1999). Approximately 10% of cases of ALS are inherited, usually as an autosomal dominant trait (Mulder et al., 1986). In ~25% of these familial ALS (FALS) cases, the disease is caused by mutations in the gene encoding cytosolic copper-zinc superoxide dismutase (SOD1) (Deng et al., 1993; Rosen et al., 1993a,b). As in sporadic ALS, FALS is manifested by degeneration of motor neurons and intraneuronal inclusions may also be seen (Ince et al., 1998). It has been hypothesized that mutations in SOD1 destabilize the folded protein, leading to neurotoxicity through mechanisms involving copper-catalyzed oxidative chemistry (hydroxyl radical formation and tyrosine nitration) (Wiedau-Pazos et al., 1996; Yim et al., 1996, 1997; Estevez et al., 1999) or protein aggregation (Bruijn et al., 1998). Subsequent work on copper-mediated chemistry, however, has not supported a role for nitration (Facchinetti et al., 1999; Doroudchi et al., 2001) or for radical formation (Singh et al., 1998; Sankarapandi and Zweier, 1999). Recent studies by Hayward et al. (2002) did not reveal a consistent abnormality in copper or zinc ion content or in specific activity of bound copper within a large set of SOD1 mutants. An earlier study also indicated an absence of influence of SOD1 activity on mutant toxicity (Bruijn et al., 1998). Importantly, in SOD1-mutant transgenic mice in which the gene encoding the copper chaperone for SOD1 (CCS) was knocked out, reduced copper loading into SOD1 did not affect the clinical development of the disease (Subramaniam et al., 2002). These observations challenge the hypothesis that the effect of SOD1 mutations in FALS is a direct result of altered copper chemistry or SOD1 activity. Relevant to this issue are studies of the thermal stability of a panel of 14 SOD1 mutants, which show that, in each case, the mutation decreased the protein's stability (Rodriguez et al., 2002). This type of destabilization could facilitate SOD1 unfolding and lead to pathologic assembly of the enzyme, a process that could explain the formation of SOD aggregates observed in transgenic mice (Bruijn et al., 1998; Johnston et al., 2000). It will be interesting to determine whether SOD1 assembly also involves prefibrillar intermediates, and if so, whether these structures are neurotoxic.

Prion Diseases

In humans, prion diseases include Kuru, Gerstmann-Straussler-Scheinker syndrome, Creutzfeldt-Jakob disease, and fatal familial insomnia (Prusiner, 1998). These diseases comprise an unusual group of progressive, fatal, neurodegenerative disorders whose etiologies may be sporadic, genetic, or infectious (Prusiner, 1998). The infectious agent, the prion, appears to be composed solely of protein. The prion protein (PrP) exists in two forms. The infectious form of PrP, termed PrP^{Sc} because of its association with the disease scrapie, can form amyloid fibrils and is partially resistant to digestion by the enzyme proteinase K (PK). A normal, cellular form, PrP^{C}, is anchored to the plasma membrane through a glycosylphosphatidylinositol linkage and is PK-sensitive. PrP^{C} is widely distributed in the body and is expressed at highest levels in neurons (Prusiner, 1998). In vivo, and recent in vitro, experiments support the hypothesis that PrP^{Sc} directs the pathologic conformational conversion of PrP^{C} into PrP^{Sc} (Cohen and Prusiner, 1998). Protein misfolding thus is a central feature of the prion diseases (Prusiner, 2001).

The structures of a number of biologically relevant forms of PrP have been solved using NMR approaches (Riek et al., 1996; Donne et al., 1997; James et al., 1997; Liu et al., 1999; Zahn et al., 2000). PrP from a variety of species shares the same overall organization, a flexible N-terminus and a globular domain comprising three α-helices and a short, anti-parallel β-sheet. During amyloidogenic assembly of PrP, a major α-helix→β-strand conversion occurs (Cohen and Prusiner, 1998). In vitro studies using a number of different recombinant PrP isoforms have revealed that oligomerization is a prominent part of the prion assembly pathway (Baskakov et al., 2000, 2002; Lu and Chang, 2001). A number of different oligomeric structures form in vitro, some of which are clearly "on-pathway" for amyloid formation and some of which may not be. When expressed in transgenic mice, however, the PrP isoforms giving rise to these oligomers all produce prion-like neuropathology (Fischer et al., 1996; Supattapone et al., 1999). The oligomerization and helix→sheet conversion events which occur during prion assembly

have also been postulated to mediate Aβ assembly (Walsh et al., 1999; Kirkitadze et al., 2001). If prion replication is a process with certain mechanistic features analogous to those of Aβ assembly, it will be important to determine if oligomeric PrP species play a role in neurodegeneration.

SUMMATION

Fibril formation has been linked with degenerative diseases since the first electron microscopic characterizations of amyloid structure in the late 1950s and early 1960s (Cohen and Calkins, 1959; Kidd, 1964; Terry et al., 1964). Recently, however, continued investigation of the folding, assembly, and biological activity of Aβ and other amyloidogenic proteins has shown that soluble, oligomeric structures form and have potent neurotoxic activity. Extracellular amyloid deposits, intracellular aggregates, (e.g., aggresomes) (Kopito, 2000), and other large protein accretions may be late-stage features of a disease process in which the most important effectors of neuronal dysfunction are soluble assemblies that act early and irreversibly (Bucciantini et al., 2002). In vitro analysis of Aβ fibril assembly has revealed a number of oligomeric intermediates, in particular, protofibrils and ADDLs. Studies of the biological activity of these oligomers in neurons, organotypic slices, and in the brains of living animals has shown that they are potent neurotoxins. In addition, in humans, protofibril formation has been postulated to cause the Arctic form of AD. More generally, protein aggregation is associated with many other neurodegenerative disorders. The pathways through which each of the specific proteins assemble into fibrils share important features with that of Aβ fibril formation. In particular, the formation of soluble, toxic oligomers may be a key pathogenetic process in these disorders, as it appears to be in AD. From a clinical perspective, research strategies enabling direct testing of this hypothesis could provide support for the targeting of fibril intermediates for medicinal chemistry approaches. It also should be recognized that, although not mentioned here, many other amyloid proteins exist that cause systemic diseases or affect other organ systems. Continued study of amyloidogenic proteins thus is likely to contribute to an improved understanding of disease pathogenesis both within and outside the nervous system. Finally, because amyloid assembly is intrinsically a protein folding problem, insights gained in the study of Aβ and related proteins should be of substantial general value.

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