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REVIEW

Diversity of Aβ deposits in the aged brain: a window on molecular heterogeneity?

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Abstract

The Aβ peptide forms a morphologically heterogeneous assortment of aggregates in the brains of patients with Alzheimer's disease. The reasons for the diversity of the histopathologically identified lesions (Aβ-plaques and cerebral β-amyloid angiopathy) are uncertain. However, there is growing evidence for the existence of higher order structural heterogeneity in protein molecules with the same amino acid sequence and differential involvement in disease. Focused analysis of plaque morphotypes could yield novel insights into the organization and function of putative protein variants in the diseased brain. In addition to classical amyloid-selective dyes, new techniques are emerging to undertake such analyses, including selective, small molecule binding agents, specific antibodies, and conformationally sensitive optical probes. By illuminating the relationships between specific lesions and their molecular components, these agents can help to clarify the complex pathology of Alzheimer's disease.

Keywords: Alzheimer's disease, amyloid, cerebral amyloid angiopathy, senile plaques, oligomers, prion, proteopathy, strains.

Introduction

A remarkable variety of neurodegenerative diseases are characterized by the aggregation of specific proteins into extracellular and intracellular deposits in the brain [1–4]. In many of these disorders, the proteins form masses of fibrillar material *in vivo* that are classically defined as *amyloid*, i.e., extracellular accumulations of proteinaceous fibrils that exhibit a cross-β structure, and therefore are birefringent upon staining with Congo Red [4, 5]. Amyloid fibrils are generally long, unbranched structures that are superficially similar, even though they can be formed from any of more than 20 different proteins in the brain and other organs [5–8]. However, even fibrils consisting of the same protein may show a degree of fibrillar polymorphism that reflects heterogeneity of the underlying polypeptide structure [4] and the conditions under which the protein polymerizes [9, 10]. Furthermore, many cerebral and systemic proteopathies involve the accumulation of proteins, both intracellularly and extracellularly, that do not fit the classical definition of amyloid [1, 5, 11]. Indeed, the *Nomenclature Committee of the International Society of Amyloidosis* recently removed the restriction that amyloid must be extracellular, defining amyloid as "an *in vivo* deposited material, which can be distinguished from non-amyloid deposits by characteristic fibrillar electron microscopic appearance, typical X-ray diffraction pattern and histological staining reactions, particularly affinity for the dye Congo red with resulting green birefringence" [8] (see also Chiti and Dobson [4] and Fändrich [12] for discussions of the definition of amyloid).

In Alzheimer's disease (AD), the most common age-associated neurodegenerative disorder, the two canonical histopathologic lesions are *senile plaques* (complex lesions characterized by extracellular deposits of fibrillar Aβ peptide) and *neurofibrillary tangles* (intracellular, fibrillar polymers of tau protein) (Figure 1). Whereas the number of tangles generally correlates more strongly with the degree of dementia than does the number of plaques [13–15], genetic, pathologic and biochemical evidence implicates the aberrant multimerization of Aβ as an early and essential event in the genesis of AD [3, 11, 16, 17]. According to this *Aβ cascade* hypothesis, neurofibrillary tangles are secondary to an initial abnormality of Aβ [3]; for this reason, much research on the origins of AD has concentrated on Aβ. Senile plaques (and a related lesion, cerebral β-amyloid angiopathy, or CAA) provide striking histological evidence of excessive protein accumulation in the AD brain, but it may be that very small soluble aggregates of Aβ known as Aβ-oligomers are the proximal cause of most neuronal damage [1, 3, 18, 19]. In any case, it is important to clarify the molecular features of Aβ that render the molecule harmful to neurons.

Paradoxically, some elderly individuals have extensive cerebral Aβ deposition yet are cognitively normal [20]. Similarly, several species of nonhuman primates, all of which generate human-sequence Aβ, manifest high levels of Aβ deposition in the brain with age [21; R.F. Rosen, unpublished observations], yet they do not become demented [22]. Furthermore, recent experiments indicate that the composition of oligomeric Aβ differs in normal and demented humans [23]. These findings and others suggest that the Aβ peptide

may comprise diverse structural/functional aggregates (or 'strains'), some of which are more toxic to neurons, and some of which are comparatively benign. Currently, however, there is largely only indirect evidence for the existence of variant strains of Aβ *in vivo*.

Molecular heterogeneity of pathogenic proteins: the strain phenomenon

In microbiology, the term "strain" is commonly used to signify structural and/or functional varieties of organisms within a given species. Mammalian prion strains, similarly, have been defined as prion protein (PrP) variants that exhibit characteristic biological properties [24, 25]. Prion diseases are progressive, incurable neurodegenerative disorders that include Creutzfeldt–Jakob disease, kuru, Gerstmann–Straussler–Scheinker disease and fatal familial insomnia in humans, as well as several diseases of nonhuman species, such as scrapie, bovine spongiform encephalopathy, and chronic wasting disease [26–28]. Histopathologically, these diseases are defined by the presence of spongiform change, neuronal loss and astrocytosis, and aggregates of PrP protein (Figure 2) with characteristic morphologies and distinct regional distributions in brain [29, 30]. The potential to generate conformationally distinct protein strains is now recognized as a common property of aggregation-prone proteins [4, 31]. Indeed, the same protein can form amyloid fibrils of varying morphology under different environmental influences, such as pH, temperature, ionic strength and protein concentration [9]. Moreover, environmental factors can influence the *in vitro* assembly of Aβ1–40 into a remarkable array of supramolecular structures [10]. These fibrillar morphotypes appear to be associated with differences in molecular packing; they can be conveyed to new fibrils in a strain-specific fashion, and the strains undergo a kind of conformational selection in which the morphotype best suited to a given environment prevails [9].

Prion disease can be transmitted from one animal to another by an extraordinary mechanism involving the structural corruption of normal, endogenous prion protein molecules (PrP^{C}) by a pathogenic conformation of PrP (PrP^{Sc}) [2, 25, 29, 32, 33]. Efficient transmission is critically dependent on characteristics of both the agent (strains) and the host [34–36]. Traits associated with prion strains can include differences in amino acid sequence, protease sensitivity, and glycosylation pattern [30], but ultimately the strain phenotype is governed by the molecular conformation of PrP [31, 37]. Because isolating and structurally characterizing the transmissible agent in prion disease has been difficult, researchers have relied on largely indirect evidence to infer the existence of pathogenic strains, such as biochemical peculiarities, incubation time, and the morphology and distribution of lesions [24].

Other cerebral proteopathies have been thought to be non-transmissible [38], but recent experiments have shown that Aβ deposition also can be induced, or seeded, in the brains of transgenic mice by Aβ-rich brain extracts [39–41]. In this model, the morphological characteristics (morphotypes) of seeded deposits depend on the features of the seeding agent and the host, reminiscent of prion strains [41, 42]. Unfortunately, it is not yet possible to generate an unambiguous representation of the 3-dimensional structure of Aβ (or PrP) in its pathogenic form(s), so the nature, and even the existence, of Aβ strains in the living brain remain uncertain. We propose that, as with ordered, crystalline aggregates of smaller molecules, the morphotypes of senile plaques may furnish clues to the underlying structure of the Aβ molecules that constitute the lesions. Furthermore, the multiplicity of plaque types suggests that several strains of Aβ may co-exist within a single brain (as can be the case in prion disease [25], see below). Biochemical and structural analysis of specific plaque types might therefore enable the separation and characterization of variant molecular species of Aβ.

In the prionoses, different strains of PrP^{Sc} often give rise to distinctive structural and regional lesion profiles in affected tissue [37]. The observation of an unusual type of lesion (the florid plaque) in humans with variant Creutzfeldt–Jakob Disease (vCJD) provided key evidence that vCJD is caused by a previously unknown prion strain [43]. Similarly, in the *in vivo* Aβ-seeding paradigm (above), different Aβ-plaque morphotypes can be generated whose appearance correlates consistently with the murine source of the Aβ-rich extract, suggestive of donor-specific, strain-like idiosyncrasies in the Aβ seeds [41]. This observation, in conjunction with evidence that soluble Aβ aggregates differ in normal aging and Alzheimer's disease [23], and can form polyfunctional assemblies that retain their properties after repeated passage *in vitro* [44], argues that Aβ is able to assume and maintain multiple, functionally variant strains. The idea of diverse molecular protein morphotypes has important implications for understanding the mechanisms by which Alzheimer's disease and other proteopathies develop and amplify in the brain. To identify and analyze naturally produced Aβ strains, it is important to isolate them from brain, since aggregates formed *in vitro* or in cultured cells lack critical characteristics of those generated *in vivo* [25, 41]. We speculate that specific types of Aβ aggregates in the diseased brain are especially pathogenic, and that they include soluble oligomeric species as well as insoluble fibrillar forms. Due to the high fidelity of molecular seeding and growth processes, similar aggregates represent a potential concentrated source of molecular variants of Aβ.

The heterogeneity of Aβ deposition in AD

The advent of sensitive and specific immunohistochemical methods for visualizing Aβ greatly expanded the known diversity of histologically identifiable protein deposits in the AD brain [45–49]. The Aβ-lesions include classical senile plaques, as well as a surprising array of Aβ-deposits of different shapes, sizes, densities and locations (Figure 3).

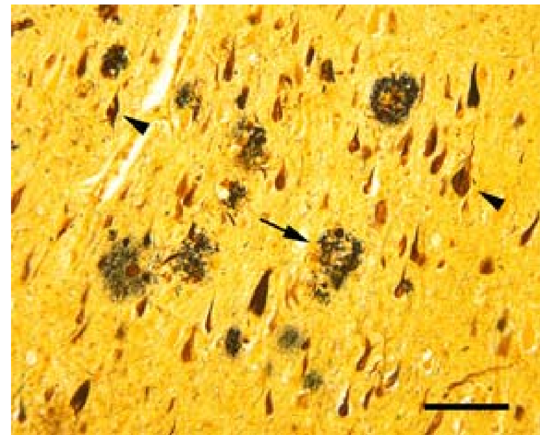


Figure 1 – *Gallyas silver-stained section from the hippocampal formation showing the canonical lesions of Alzheimer's disease: senile plaques (one designated by the arrow) and neurofibrillary tangles (2 marked by arrowheads). Bar = 100 µm*

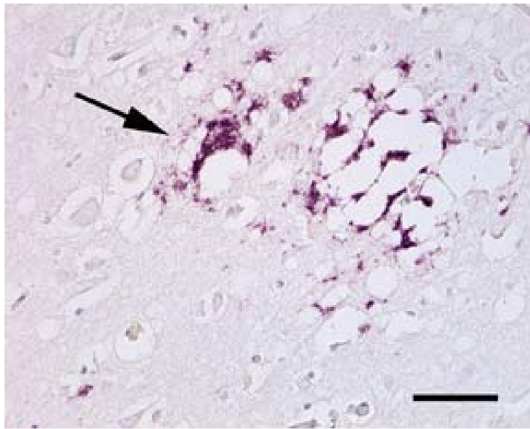


Figure 2 – *Prion protein accumulation and spongiform degeneration (arrow) in a case of Creutzfeldt–Jakob disease. The degree of deposition and morphotypes of the deposits can vary in different manifestations of prion disease. Case courtesy of Professor Rolf Warzok, University of Greifswald. Antibody 3F4 to PrP. Bar = 50 µm*

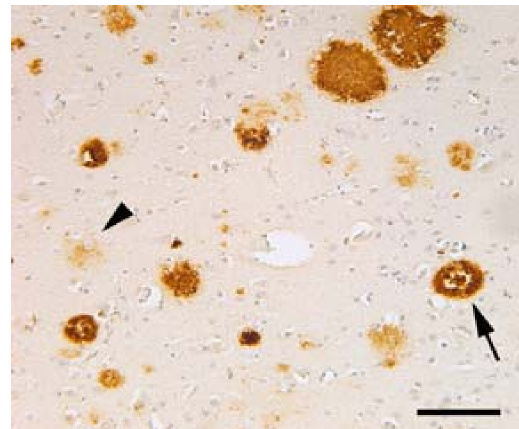
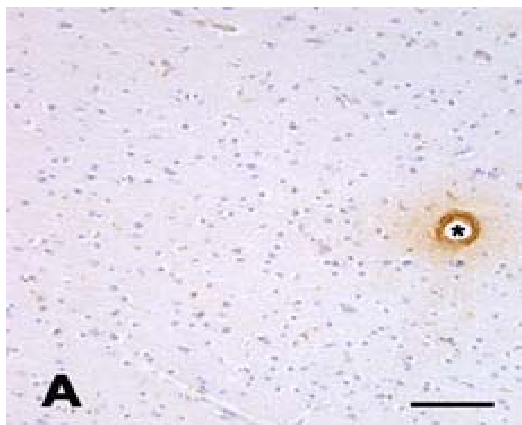


Figure 3 – *Heterogeneity of parenchymal Aβ-deposits ('plaques', in the broad sense of the term) in the neocortex of a case of Alzheimer's disease. The arrow denotes a senile plaque with a core-space-shell Aβ-structure; the arrowhead marks a diffuse deposit of Aβ. Many other sizes and shapes of Aβ-immunoreactive lesions also are evident. Antibody 6E10 to Aβ, Nissl counterstain. Bar = 100 µm*



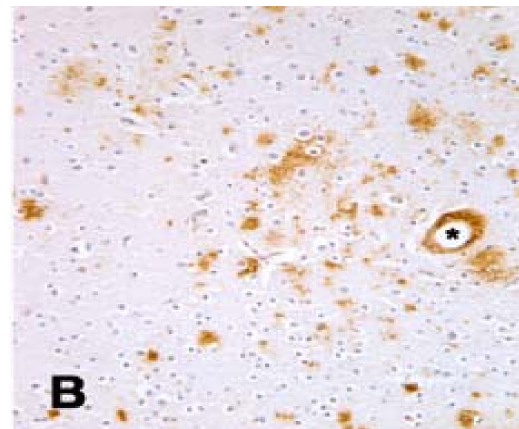


Figure 4 – *Differential staining of Aβ deposits by antibodies to Aβ40 (A) and Aβ42 (B) in nearby neocortical sections from a case of Alzheimer's disease. Asterisks denote the same blood vessel. Note that diffuse parenchymal deposits in this case are immunoreactive almost exclusively for Aβ42, whereas vascular Aβ is immunoreactive for Aβ40 and Aβ42. Antibodies R163 to Aβ40 and R165 to Aβ42 courtesy of Dr. Pankaj Mehta. Bar = 100 µm for both A and B*

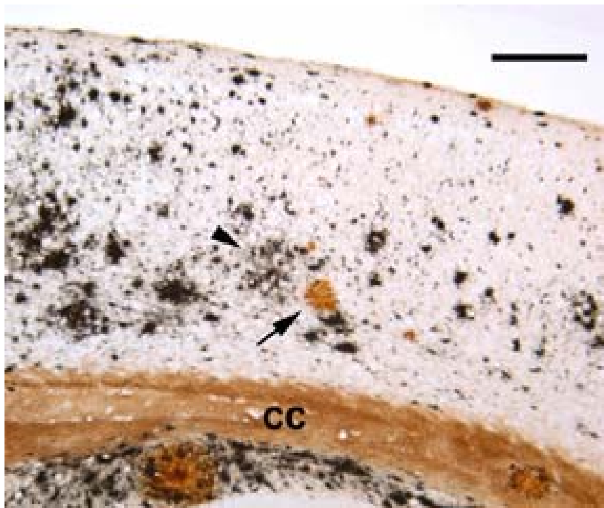


Figure 5 – ***Campbell–Gallyas silver-stained section showing Aβ deposition in a portion of the neocortex (dorsal) and hippocampus (ventral to the corpus callosum [CC]) of a 16 month-old Tg2576 transgenic mouse overexpressing human β-amyloid precursor protein with the 'Swedish' mutation (APPSwe) [74]. Note the variety of diffuse deposits (black, one denoted by the arrowhead) and a small number of dense plaques that are stained golden-brown (arrow). Different types of transgenic mice generate different lesion phenotypes. Bar = 200 µm***

The morphology of Aβ deposits is influenced by the architectonic features of the region in which they appear [46], and possibly also by local biochemical idiosyncrasies. However, it is not unusual to see a variety of plaque types intermixed within a given cortical locale (Figure 3), suggesting that highly localized factors may be important. Clues that these diverse lesions may not be simply different stages along a pathway to end-stage, compact Aβ-amyloid deposits include the observation that they are differentially immunoreactive with antibodies to discrete segments of Aβ [49] (Figure 4), and that some 'diffuse' protein deposits (such as the amorphous cloud of Aβ in the entorhinal cortex) apparently never evolve into classical senile plaques [46]. Because environmental conditions that affect molecular packing also influence the morphology of Aβ (and other) amyloid fibrils [9], the conditions under which the seed for a given plaque is first generated (e.g., intracellular or extracellular?) could have a decided influence on the ultimate structure of the lesion.

Recent evidence that soluble Aβ-oligomers differ from those that arise during normal aging, both in terms of cytotoxicity and aggregation [23], suggests that different molecular conformations of Aβ also might yield different plaque types in brain (and perhaps that some conformations may not produce histologically identifiable plaques at all). It is instructive to note as well that, while Aβ deposition in aged APP-transgenic mice can be quite copious and diverse (Figure 5), these mice do not exhibit the full behavioral or pathologic phenotype of AD. It appears that the characteristics of murine Aβ aggregates differ in important ways from those in AD, including post-translational modifications, solubility [50], and a paucity of high-affinity binding sites for certain amyloid binding agents (below) [51, 52].

Analyzing the molecular diversity of Aβ in specific lesions

To ascertain the diversity of Aβ multimers in brain, it will be informative to determine where, how and why strain-like pathomolecular differences exist. Examination of brain tissue confirms the presence of truncated Aβ peptides *in vivo* [53–55], but how these fragments are compartmentalized in the diseased brain remains incompletely understood. Spatial isolation of diverse senile plaques, as well as capillary- and large vessel-CAA, allows us to focus on defined lesions for biochemical and biophysical investigations. One approach is to isolate distinct subtypes of senile plaques or CAA from tissue sections via biochemical fractionation or laser-capture microdissection. These selectively enriched lesion preparations can then be analyzed for the presence of different fragments or post-translational modifications of Aβ by mass spectrometry, or for the presence of auxiliary components of the lesions [12, 56–59]. Another approach is the *in situ* examination of intact lesions within tissue sections. In addition to classical dyes such as Congo Red and Thioflavin, sequence- or conformation-selective antibodies are useful for differentiating lesion types [60–66]. Analysis with Aβ-binding radioligands such as PIB [51, 52] also can be informative, and such agents currently are being used to image Aβ deposition in living subjects [e.g., 67]. Biophysical methods such as Fourier-transform infrared spectroscopy (FTIR) for assessment of β-sheet content can be applied to tissue sections, albeit with limitations [12]. NMR microscopy can resolve moderate-sized plaques in postmortem tissue samples and report on plaque-induced effects on water structure in the immediate locale in living transgenic mice [68, 69]. An exciting new possibility for detecting structurally variant molecules within identified lesions is the use of novel orientation- and packing-sensitive luminescent probes [70, 71]. Different conformational states of Aβ-amyloid fibrils generated *in vitro* and *in vivo* can be distinguished optically as a result of subtle changes in the structure of flexible luminescent conjugated polyelectrolyte probes (LCPs) [70]. Such markers could facilitate the rapid and sensitive assessment of structural differences in defined Aβ aggregates in tissue sections.

Finally, the analysis of strain-like behavior of aggregation-prone proteins *in vivo* can be complemented by studies of nucleated protein aggregation *in vitro*. An interesting model is the generation of large, plaque-like spherulites on surfaces by the seeded growth of amyloid protein polymers [10, 72]. (In polymer science, spherulites are semi-crystalline, spherical collections of linear polymers). The amyloid spherulite paradigm enables that controlled growth of supramolecular protein assemblies and a systematic examination of the conditions under which

they develop. Surface features can influence the nature of the polymers, and polymerization can be faithfully transmitted to naive molecules by defined seeds in a strain-like fashion [10]. Selection of structurally defined polymers might expedite the isolation of purified strains for *in vivo* seeding experiments, an important step in fulfilling Koch's postulates for characterizing the agent in transmissible proteopathies [36].

In Alzheimer's disease brain, the Aβ peptide forms an extensive variety of lesions, the morphological characteristics of which may reflect the multidimensional structural features of their primary molecular components and which also may serve as a link to specific disease processes. New analytical methods are evolving that allow a more informative analysis of the makeup of plaques *in situ* than has been possible in the past. The emergence of reliable and accurate techniques for the measurement of prefibrillar (oligomeric) aggregates in tissue samples will contribute greatly to our knowledge of the pathogenicity of aggregated proteins. Future studies should be directed toward understanding the reasons why proteins assume different multimeric forms. This may provide clues as to why these particular species are pathologic, or, alternatively, how they are indicators of pathologic processes.

Is a particular fragment (or fragments) of Aβ particularly pathogenic, or is the functionality of each assembly defined by a different combination of fragments? What role do post-translational modifications play in modulating protein aggregation? How do other factors contribute to structural variation, such as pH, temperature, physical shear forces and macromolecular crowding [9, 73]? Is it possible to identify, and perhaps measure, specific types of assembly for purposes of diagnosis and treatment? These investigations will provide insights into the generation of pathogenic protein assemblies, help to guide the development of imaging agents and therapeutic interventions, and may also clarify the uniquely human predisposition to Alzheimer's disease.

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