

## New and Notable

### Sorting Out the Driving Forces for Parallel and Antiparallel Alignment in the A $\beta$ Peptide Fibril Structure

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Protein misfolding and aggregation are now well-recognized processes that often lead to amyloid fibril formation (amyloidosis). Because these events are coupled with many types of human disease, the field of protein amyloidosis is under intense investigation. In the amyloid fibrils, the proteins adopt cross  $\beta$ -pleated sheet structures with distinct tinctorial and morphological properties, and typically consist of long, unbranched filaments that bind to diagnostic dyes such as Thioflavin-T and Congo Red (Temussi et al., 2003). There are currently 24 proteins that produce amyloid fibrils associated with human disease (Westermarck et al., 2002), including the A $\beta$  of Alzheimer's disease, prion of transmissible spongiform encephalopathies, amylin of maturity-onset diabetes, transthyretin of familial amyloidosis, huntingtin of Huntington's disease, and  $\alpha$ -synuclein of Parkinson's disease. Albeit these proteins have very different primary sequences, molecular sizes, and folded tertiary structures, they all produce amyloid fibrils. Amyloidosis may also be a general property for all proteins, since de novo designed peptides and other naturally occurring proteins not associated with human disease (such as myoglobin) (Fandrich et al., 2001) can be encouraged to aggregate as amyloid fibrils.

Despite the plethora of research, details about the molecular mecha-

nisms of amyloidosis are still lacking. Most scientists agree that unraveling the chemical mechanisms is absolutely essential for the development of specific inhibitors to prevent amyloidosis in humans. The most obvious missing details are high-resolution structural data, particularly regarding the soluble  $\beta$ -sheet aggregates and the amyloid fibril structures. Given that amyloid fibrils are not amenable to standard x-ray crystallography (i.e., they do not form crystals), lower-resolution analytical techniques such as x-ray fibril diffraction, negative stain electron microscopy, and atomic force microscopy have been employed. Although these methods have provided valuable data about the fibril morphology, including the discovery of "toxic" intermediates such as the "protofibrils" (Caughy and Lansbury, 2003), they cannot provide critical atomic level structural information, such as what amino acids are interacting during the aggregation processes and whether or not the fibrils adopt unique, folded structures. In this regard, the work presented by the Tycko and Meredith research groups in this issue makes an important contribution about the A $\beta$  fibril structure of Alzheimer's disease. These research groups have excellent track records with handling the A $\beta$  peptide and were the first to demonstrate that the peptide adopts the rare parallel  $\beta$ -sheet organization in the amyloid fibrils.

The recent finding that solid-state NMR spectroscopy is applicable to amyloid fibril structure determination represents a timely and significant breakthrough (Tycko, 2003). For biomolecules, solid-state NMR has unique capabilities, in that it can be used with samples of limited solubility that often precipitate as noncrystalline solids. Solid-state NMR can provide accurate distances and torsion angles between site-specific  $^{15}\text{N}$ - and/or  $^{13}\text{C}$ -labeled atoms, and, under certain conditions, the NMR constraints can generate structural models on par with those obtained by solution NMR and x-ray.

Solid-state NMR is likewise a rapidly growing field, and studies of uniformly or specifically labeled samples have provided important information about membrane-bound peptide channels and the active sites of other membrane proteins (Thompson, 2002), both of which are systems not amenable to solution NMR or x-ray.

The predominate forms of the Alzheimer's A $\beta$  peptide are the 40-residue A $\beta$ (1–40) and 42-residue A $\beta$ (1–42). The earliest solid-state NMR studies utilized peptide fragments composed of regions important for amyloidosis, such as the A $\beta$ (34–42) and A $\beta$ (16–22) peptides, in which both form the antiparallel  $\beta$ -sheet as the major structural motif. However, solid-state NMR studies of the longer 35-residue A $\beta$ (10–35) and native A $\beta$ (1–40) peptides found different results that were consistent with in-register parallel  $\beta$ -sheet structures. The overall conclusion was that accurate structural models must use constraints obtained from the longer, full-length A $\beta$  peptides, and that despite the common cross  $\beta$ -pleated sheet motif, specific structural details can be sequence dependent. Nonetheless, the parallel versus antiparallel variation was a conundrum, since the shorter A $\beta$  peptides still form classic amyloid fibrils based on Congo Red and electron microscopy.

As described in this issue, Gordon et al. contemplated that peptide amphiphilicity may influence the parallel versus antiparallel orientation. Inspection of the A $\beta$  primary sequence reveals that the A $\beta$ (16–22) and A $\beta$ (34–42) peptides are nonamphiphilic, whereas the A $\beta$ (10–35) and A $\beta$ (1–40) are amphiphilic. Additionally, the A $\beta$ (1–40) peptide has surfactant properties and forms micelles. Amphiphilicity would stabilize the parallel  $\beta$ -sheet orientation and nonamphiphilicity the antiparallel, possibly by way of hydrogen-bonding or electrostatic interactions between oppositely charged side chains or termini.

Submitted October 21, 2003, and accepted for publication October 27, 2003.

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0006-3495/04/01/01/02 \$2.00

To test this hypothesis, the A $\beta$ (16–22) peptide was made amphiphilic by attaching a long hydrophobic chain to one end of the molecule (Scheme 1). This objective was fulfilled by acylation of the N-terminus with octanoic acid (CH<sub>3</sub>(CH<sub>2</sub>)<sub>6</sub>COOH), which increased the peptide's amphiphilicity as measured from its stability at the air-water interface, but did not reduce its ability to form amyloid fibrils. Based on electron microscopy and Congo Red, the modified octanoyl-A $\beta$ (16–22) forms amyloid fibrils that are indistinguishable from those of unmodified A $\beta$ (16–22). Without a doubt, the hypothesis was proven accurate by solid-state NMR with peptides containing <sup>13</sup>C- and <sup>15</sup>N-labels at strategic sites, where the octanoyl-A $\beta$ (16–22) formed parallel  $\beta$ -sheets and the A $\beta$ (16–22) antiparallel  $\beta$ -sheets. For consistency, the NMR methods employed were identical to those used previously by the same group for generating the A $\beta$ (1–40) peptide model

(Petkova et al., 2002), which included a combination of low-precision (line-widths and chemical shifts) and high-precision (distances and torsion angles) constraints. The labeling and dilution strategy utilized four different peptide mixtures and exemplifies the experience these groups have in studying the A $\beta$  by solid-state NMR.

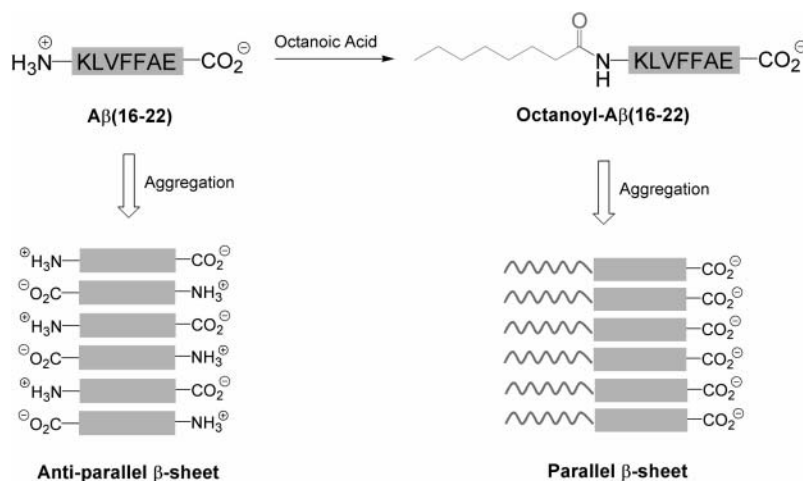
The work of Gordon et al. in this issue has several important implications; notably, that peptide amphiphilicity is a critical parameter for controlling the  $\beta$ -sheet organization of amyloid fibrils. This provides a rationale for the proclivity toward parallel or antiparallel arrangements and, more importantly, that amyloid-forming proteins may each adopt unique fibril structures that are only visible by high-resolution techniques such as NMR. The unique fibril structures and the importance of amphiphilicity suggest that specific compounds could be targeted toward inhibiting amyloidosis of a single or select group of proteins,

possibly by altering the brain micro-environment in a manner to prevent formation of the native parallel arrangement. Related approaches with modulating the peptide amphiphilicity has been used in the design of tertiary and supramolecular structures and may also be useful in the development of self-assembling, nanoscale materials.

In closing, the article by Gordon et al. in this issue clearly shows that high-resolution structure determination is urgently needed in the amyloid research area, and that solid-state NMR has emerged as an indispensable tool in this endeavor.

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SCHEME 1