Nicotine and Amyloid Formation

Hong Zeng, Yongbo Zhang, Li-Jun Peng, Haiyan Shao, Nanda K. Menon, Jing Yang, Arthur R. Salomon, Robert P. Freidland, and Michael G. Zagorski

The major protein constituents of amyloid deposits in Alzheimer's disease (AD) are the 40-residue β-amyloid (Aβ) (1–40) peptide and the 42-residue Aβ(1–42) peptide. The Aβ(1–42) is more pathogenic and produced in greater quantities in familial forms of AD. A major goal of research is to uncover a suitable inhibitor that either slows down or inhibits Aβ formation (β-amyloidosis). During β-amyloidosis, structural changes associated with the conversion of monomeric Aβ peptide building blocks into the aggregated fibrillar β-sheet structures occur (α-helix→β-sheet or random, extended chain→β-sheet). In previous work, we and others established that nicotine, a major component of cigarette smoke, inhibits β-amyloidosis of the Aβ(1–42), which may result from nicotine binding to the α-helical structure. These conclusions were based on solution nuclear magnetic resonance (NMR) spectroscopic studies with the nonnative 28-residue Aβ(1–28). This information suggests that, when administered therapeutically to AD patients, nicotine may not only affect cholinergic activation, but could also conceivably alter amyloid deposition. In this report, NMR studies were augmented with the naturally occurring Aβ(1–42), under conditions where the peptide folds into a predominantly α-helical or random, extended chain structure. The major result is that nicotine shows only modest binding to these conformations, indicating that the nicotine inhibition to β-amyloidosis probably results from binding to a small, soluble β-sheet aggregate that is NMR invisible. Biol Psychiatry 2001;49:248–257 *© 2001 Society of Biological Psychiatry*

Key Words: Amyloid, Aβ peptide, nicotine, NMR, Alzheimer's disease

Introduction

Alzheimer's disease (AD) is the major cause of adult-onset dementia and is characterized by an abundance of intraneuronal neurofibrillary tangles and extracellular amyloid plaques (Iqbal et al 1999; Selkoe 2000). The major component of the amyloid plaques is the β-amyloid (Aβ), a normally secreted small peptide that exists in two predominant forms: 1) the 40-residue Aβ(1–40) and 2) the 42-residue Aβ(1–42) (Figure 1). The Aβ(1–40) and Aβ(1–42) peptides differ in the absence or presence of two extra C-terminal residues. Both peptides result from the processing of a larger amyloid precursor protein (APP) (Hardy 1997) (Figure 1). The amyloid deposits are characterized by distinct tinctorial properties and fibrils of 7–10 nm in diameter, in which the primary components are aggregated proteins with antiparallel cross–β-pleated sheet structures (Malinchik et al 1998; Teplow 1998).

The biological functions of both the APP and the Aβ are currently unknown, although they are believed to play roles in neuronal homeostasis, cell adhesion, G protein coupling, and/or oxidative stress. Numerous genetic and cell viability studies support a key role for the Aβ peptide in AD neurodegeneration. The Aβ peptide becomes neurotoxic to cortical cell cultures when aggregated as amyloidlike β-sheet structures (Geula et al 1998; Iversen et al 1995; Pike et al 1995; Simmons et al 1994). Biochemical studies also suggest that the longer, 42-residue Aβ(1–42) is more pathogenic than the shorter 40-residue Aβ(1–40) (Younkin 1995), due to its greater in vitro tendency to aggregate and precipitate as amyloid (Barrow and Zagorski 1991). Recent studies demonstrated that the Aβ(1–42) is significantly elevated in cerebrospinal fluid of AD patients (Andreasen et al 1999; Jensen et al 1999; Kuo et al 1999; Wang et al 1999).

On the basis of extensive biophysical measurements, in solution the Aβ peptide can fold into α-helical or random, extended chain structures, as well as soluble β-sheet structures that eventually precipitate as amyloid (Huang et al 2000; Teplow 1998; Zagorski 1999). The relative ratios of the structures are strongly dependent on the solution conditions, with hydrophobic lipidlike environments encouraging α-helical structure and high ionic strength and midrange pH 4–7 values favoring β-sheet. The α-helical and random, extended chain structures are monomeric, whereas the late-formed β-sheet structures in solution are aggregated (oligomeric) and neurotoxic (Lambert et al 1998; Sayre et al 1997). Because the β-sheet structure is

From the Department of Chemistry (HZ, YZ, L-JP, HS, JY, ARS, MGZ) and the Department of Neurology, School of Medicine (RPF), Case Western Reserve University, Cleveland, Ohio, and the Department of Biochemistry, University of Georgia, Athens (NKM).

Address reprint requests to Michael G. Zagorski, Case Western Reserve University, Department of Chemistry, Cleveland OH 44106-7078.

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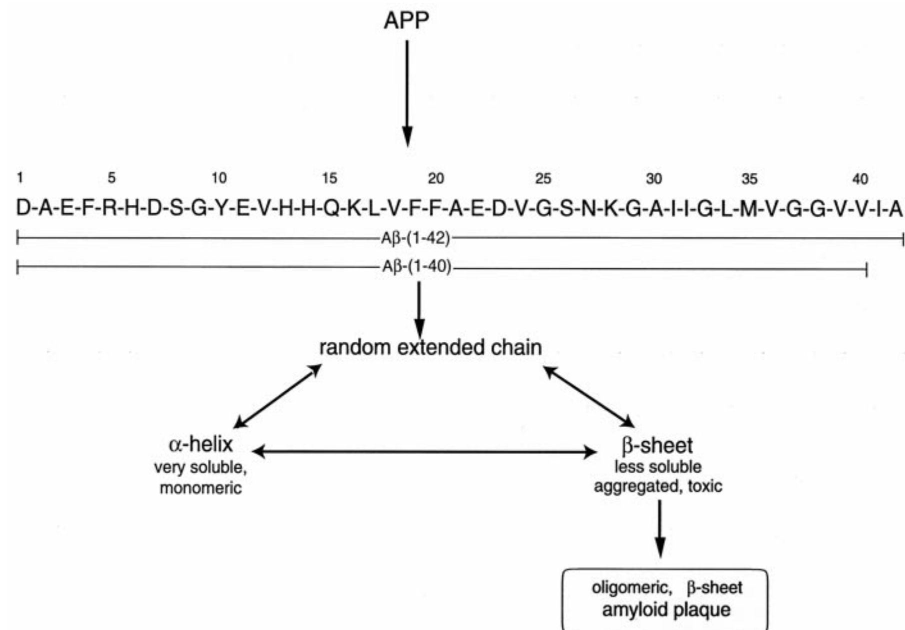


Figure 1. Overview of the formation of the β-amyloid (Aβ) peptide from the amyloid precursor protein (APP), including the amino acid sequences for the Aβ(1–40) and Aβ(1–42) peptides. Depending upon conditions, the Aβ peptide exists in distinct conformations in solution, but in the amyloid deposit, only the oligomeric β-pleated sheet structure is present. Both peptides can aggregate into soluble β-sheet structures, which are neurotoxic and eventually precipitate as amyloid.

neurotoxic, a therapeutically useful inhibitor should bind or stabilize the α-helical, random, extended chain, or early formed β-sheet structures, which are very soluble and nontoxic.

Nicotine is a predominant component of cigarette smoke and is currently being used in pilot clinical studies for the treatment of AD (Emilien et al 2000; Wilson et al 1995). The beneficial effects of this treatment have been attributed to an upregulation of nicotine receptors that are deficient in the AD brain, or possibly a protection from the Aβ-induced neurotoxicity (Kihara et al 1997; Zamani et al 1997). We and others have shown that nicotine may have a dual effect, in that besides promoting the upregulation of receptors or being neuroprotective, it may also inhibit β-amyloidosis. These conclusions were formulated on in vitro studies that established that nicotine slows down Aβ(1–42) fibril formation (Moore et al 2000; Salomon et al 1996) and that this inhibition may be due to binding to the α-helical structure (Zagorski 1999). The latter was based on nuclear magnetic resonance (NMR) studies of the homologous Aβ(1–28) peptide that contains residues 1–28 of the Aβ(1–42). Here we report an extension of this work with the Aβ(1–42) and show that the inhibition does not result from binding to either the α-helical or random, extended chain structures.

Methods and Materials

Sample Preparation

Distilled/deionized water, nicotine, and cotinine were of the highest grade possible from commercial sources and were used without further purification. The organic solvents trifluoroacetic acid (TFA) and hexafluoroisopropanol (HFIP) were distilled under an inert atmosphere of nitrogen and stored in opaque bottles at 5°C. The perdeuterated sodium dodecyl sulfate (SDS-d_{25}), EDTA (Na_2EDTA-d_{12}), 3-(trimethylsilyl)propionate-2,2,3,3-d_4 (TSP), and the solvent deuterium oxide (D_2O) were obtained from Cambridge Isotopes (Andover, MA).

The synthetic Aβ peptides were obtained from commercial sources (Anaspec, San Jose, CA) or prepared using standard Fmoc chemistry on an automated (PerSeptive Biosystems [Foster City, CA] 9050-Plus) synthesizer. Uniformly (>95%) ^{15}N-labeled Aβ(1–42) were prepared biosynthetically from *Escherichia coli* as a recombinant fusion protein in minimal media containing $^{15}NH_4Cl$ as the sole nitrogen source. The peptide was cleaved from the fusion protein at pH 8.3 using restriction protease Factor Xa. To isolate the cleaved Aβ(1–42) from the fusion protein components, aggregation of the Aβ(1–42) was induced by stirring in water (12 hours, pH 4–7). The aggregated Aβ(1–42) was collected by centrifugation (10,000 *g,* 20 min) and purified by reverse-phase high-performance liquid chromatography, with either a Zorbax-300 Bonded Silica (Rockland Technologies) or a Vydaq-259VHP822 column (Separation Group). The solvent system consisted of a linear gradient of

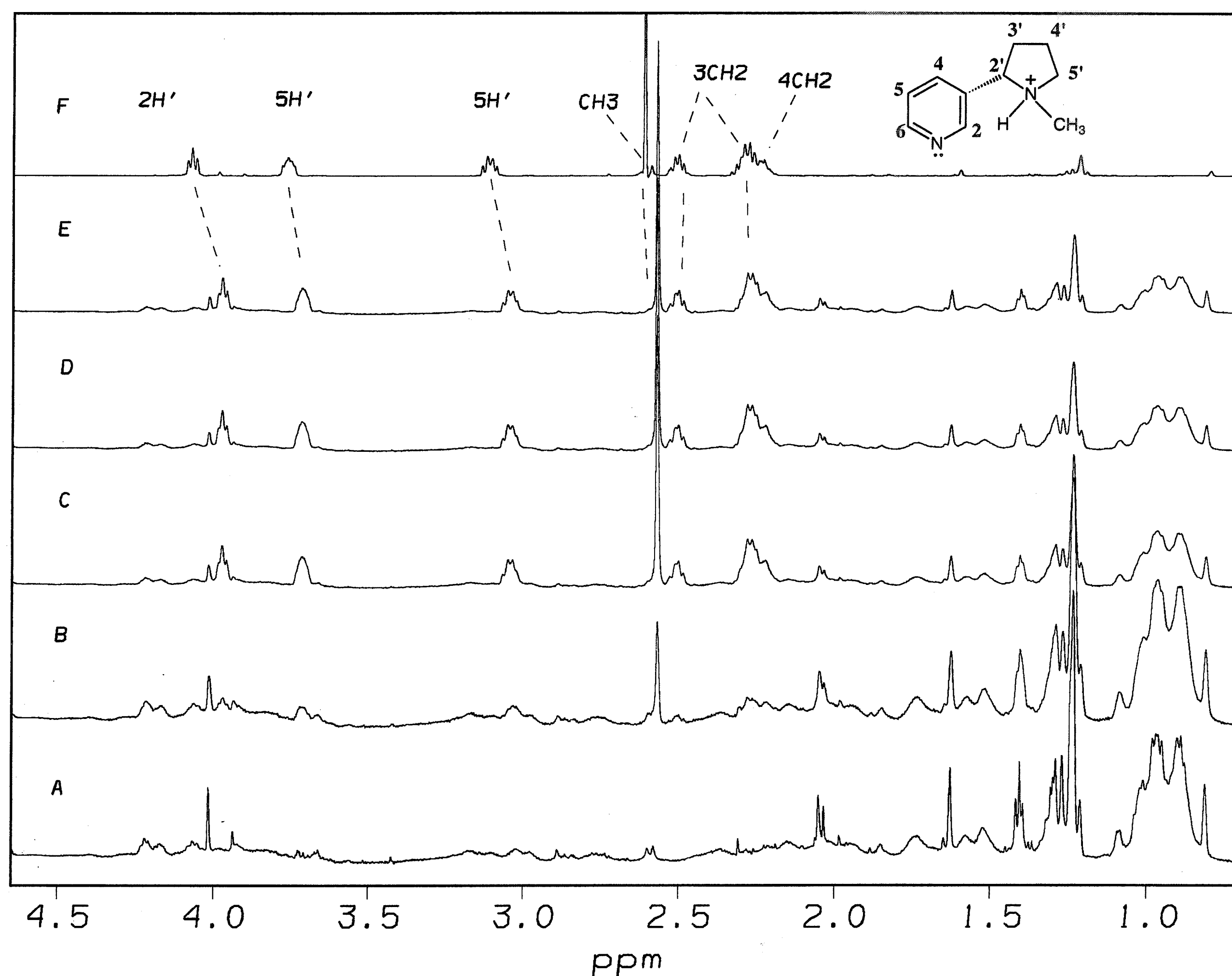
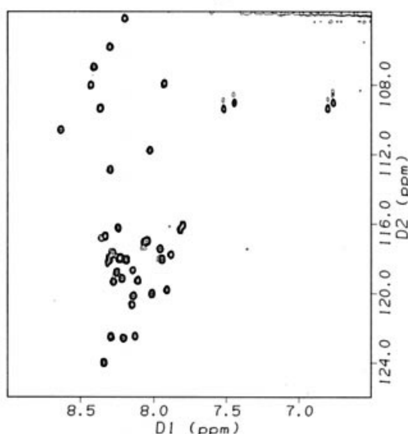


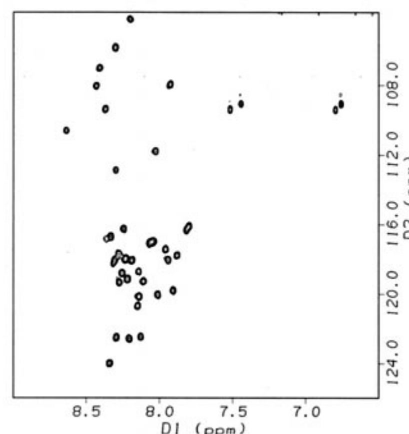
Figure 2. Upfield regions of the proton nuclear magnetic resonance (^1H NMR) spectra (600 MHz) for the β-amyloid (Aβ) (1–42) **(A)** alone (0.5 mM) and with **(B)** 0.25 mM, **(C)** 0.50 mM, **(D)** 1.0 mM, and **(E)** 2.0 mM nicotine. The upper spectrum **(F)** corresponds to nicotine without the Aβ(1–42). All solutions contained 80 mM sodium dodecyl sulfate (SDS-d_{25}) and 20 mM phosphate buffer at pH 7.2 and 25°C. The NMR peaks for nicotine are shown with the assignments.

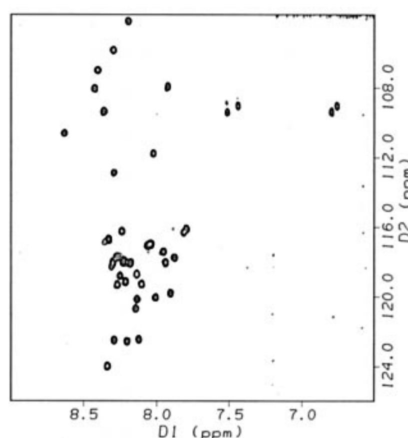
20–80% acetonitrile in water that contained either 0.1–0.08% TFA or a sodium acetate buffer (5 mmol/L) at pH 8.0, which was heated to 55–60°C to improve peak resolution and purity (Boyes 1995). Peptide identity, verified by mass spectrometry and NMR, had purity levels greater than 90%.

To disaggregate the Aβ(1–42) and generate exclusively monomeric random, extended chain structure, the purified peptides were predissolved in neat TFA solution (Jao et al 1997; Zagorski et al 1999). Trace amounts of TFA were removed by redissolving in HFIP (5:1 [mg:mL]) and then removing the HFIP with N_2 and vacuum (0.5 mm Hg, 2 hours). The disaggregation afforded by TFA allows the dry, unstructured, and monomeric Aβ to fold into its nativelike conformation in water, without potential interferences from trace organic cosolvents or small aggregates ("seeds") of the Aβ.

The Aβ(1–42) peptide solutions (0.60 mL) were prepared in 5-mm NMR tubes at concentrations of 0.1–1.4 mmol/L. The solvent-free, TFA-pretreated peptides were dissolved in either SDS-d_{25} solution in H_2O (for studies examining the effects of nicotine on the α-helical structure) or in 9:1 H_2O:D_2O (for studies examining the effects of nicotine on the random, extended chain structure). Stock solutions of (*S*)-(−)-cotinine, (*R*)-(+)-nicotine, and (*S*)-(−)-nicotine were prepared in buffered water (pH 7.2) at 20 mmol/L concentrations, after which aliquots were added to the NMR solutions containing the Aβ(1–42). All solutions contained 20 mmol/L sodium phosphate buffer at pH 7.2, 0.05 mmol/L Na_2EDTA-d_{12}, 0.05 mmol/L sodium azide (NaN_3), and 0.05 mmol/L TSP. The latter three components were included to remove trace metal ions, prevent microbial growth, and provide an internal chemical shift reference at 0 ppm, respectively. The SDS-d_{25} concentration was kept high relative to the peptide concentration so that it was well above 8 mM (the







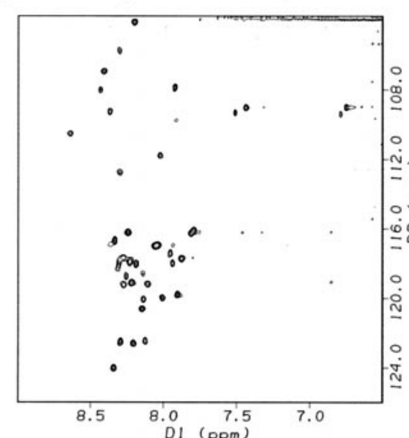


Figure 3. The two-dimensional ^{1}H-^{15}N heteronuclear single-quantum coherence spectra of recombinant ^{15}N-labeled β-amyloid (Aβ) (1–42) (0.20 mM) in 9:1 H_2O:D_2O phosphate buffer at pH 7.2 and 5°C. A single sample was separated into four parts to which 0.40 mmol/L of (*S*)-(−)-cotinine, (*R*)-(+)-nicotine (the inactive enantiomer), or (*S*)-(−)-nicotine (the active enantiomer) was added. The spectra for 0.40 mmol/L of (*S*)-(−)-cotinine and (*R*)-(+)-nicotine were identical to that of the Aβ(1–42) alone; however, the spectra containing (*S*)-(−)-nicotine showed minor perturbations (see expanded plot in Figure 4).

critical micelle concentration) and also above the average aggregation number (Henry and Sykes 1994). To ensure that the buffer maintained pH 7.2 during the titrations, the pH of the solutions was checked with a special pH electrode (Microelectrodes) that fit inside the 5-mm NMR tube. The pH values were measured at room temperature and corrections for isotope effects or for the presence of SDS-d_{25} were not performed, since control experiments showed that these substances did not significantly alter the pH.

Nuclear Magnetic Resonance Spectroscopy

All NMR spectra were acquired at 600 MHz using a Varian Inova-600 spectrometer. The NMR data were transferred to Indigo XS24 (Silicon Graphics) computer workstations and processed using the FELIX (version 97, Biosym) program. Chemical shifts were referenced to an internal standard of TSP. The one-dimensional NMR spectra were acquired with a presaturation pulse that was applied during the recycle delay (2 sec) to suppress the H_2O signal. Typically, most one-dimensional spectra had 8000-Hz spectral widths and 32-K complex data points. To further enhance the digital resolution and the signal to noise, before Fourier transformation the data were zero filled once and multiplied by a Lorentzian-to-Gaussian window function.

The two-dimensional ^{1}H-^{15}N heteronuclear single-quantum coherence (HSQC) experiments were recorded with a uniformly ^{15}N-labeled Aβ(1–42) sample with 32 scans and 1024 complex points and the transmitter placed on the water signal (Bax and Grzesiek 1993). The sweep widths were 6373.5 and 2000.0 Hz in the F_2 and F_1 dimensions, respectively. Phase-sensitive data

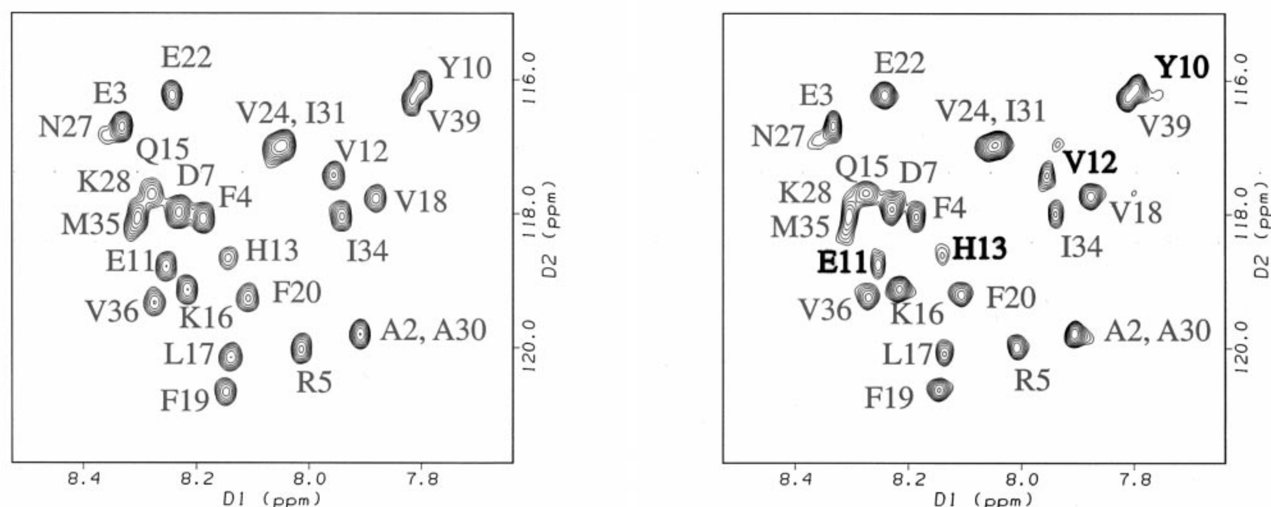


Figure 4. The expanded ^{1}H-^{15}N heteronuclear single-quantum coherence spectra of recombinant ^{15}N-labeled β-amyloid (Aβ) (1–42) [0.20 mmol/L alone and with (*S*)-(−)-nicotine (0.60 mmol/L)] (for complete spectrum, see Figure 3). Note that peaks of Tyr10, Glu11, Val12, and His13 become reduced in intensity with nicotine and the Val12 peaks splits into a doublet.

were collected using time-proportional phase incrementation in the F_1 dimension, and pulsed field gradients were employed to minimize the artifact content of the spectra, suppress the intense H_2O resonance, and select for the coherence transfer pathway, whereby magnetization passes from ^{15}N to ^{1}H for observation (Kay 1995).

Results

The NMR approach has the distinct advantage of being able to sequence-specifically determine where a particular secondary structure is located within the primary sequence. Furthermore, for drug design the NMR method can provide valuable information about protein dynamics and specific data about the individual amino acid side-chains that bind to a particular ligand (Craik 1996; Hajduk et al 1999; Shuker et al 1996). With sufficient distance and dihedral angle constraints, the NMR approach is also capable of rendering a complete three-dimensional structure.

Our sample preparation protocol ensures that the Aβ(1–42) peptide adopts a well-defined, monomeric state before starting the NMR measurements. A major difficulty relates to the ease in which the Aβ(1–42) aggregates and precipitates, which in turn creates problems in the reiteration of results. Because the peptide is very prone to time-dependent aggregation (Zagorski et al 1999), all preaggregated peptide material was carefully removed from the samples before analysis (Jao et al 1997). After this disaggregation was performed, the Aβ solutions were stable for several days at pH 7.2 and 5°C, with no precipitation or other spectral changes detected by NMR. Nuclear magnetic resonance diffusion and sedimentation equilibrium experiments confirmed that the Aβ(1–42) adopts intact, nondegraded monomeric states (Shao et al, submitted).

NMR Studies with the α-Helical Structure

Previous studies established that the Aβ(1–28), Aβ(1–40), and Aβ(1–42) peptides fold into predominantly monomeric α-helical structures in lipid environments (Coles et al 1998; Marcinowski et al 1998; Shao et al 1999; Talafous et al 1994; Terzi et al 1997). Because solution NMR studies with high-molecular-weight lipids (i.e., bilayers) are not feasible (due to slow tumbling rates), detergents such as the negatively charged SDS micelle are commonly used. Detergent micelles adequately mimic a membranelike environment and are frequently used in the structural studies of peptides and proteins (Henry and Sykes 1994). Studies of the Aβ in lipidlike environments are important and may be pertinent to the native state, when bound to lipoproteins and albumin in human plasma (Biere et al 1996). The present studies were undertaken to explore the effect of nicotine on the α-helical structure of the Aβ(1–42) peptide in SDS solution.

Our previous NMR studies of the Aβ(1–28) demon-

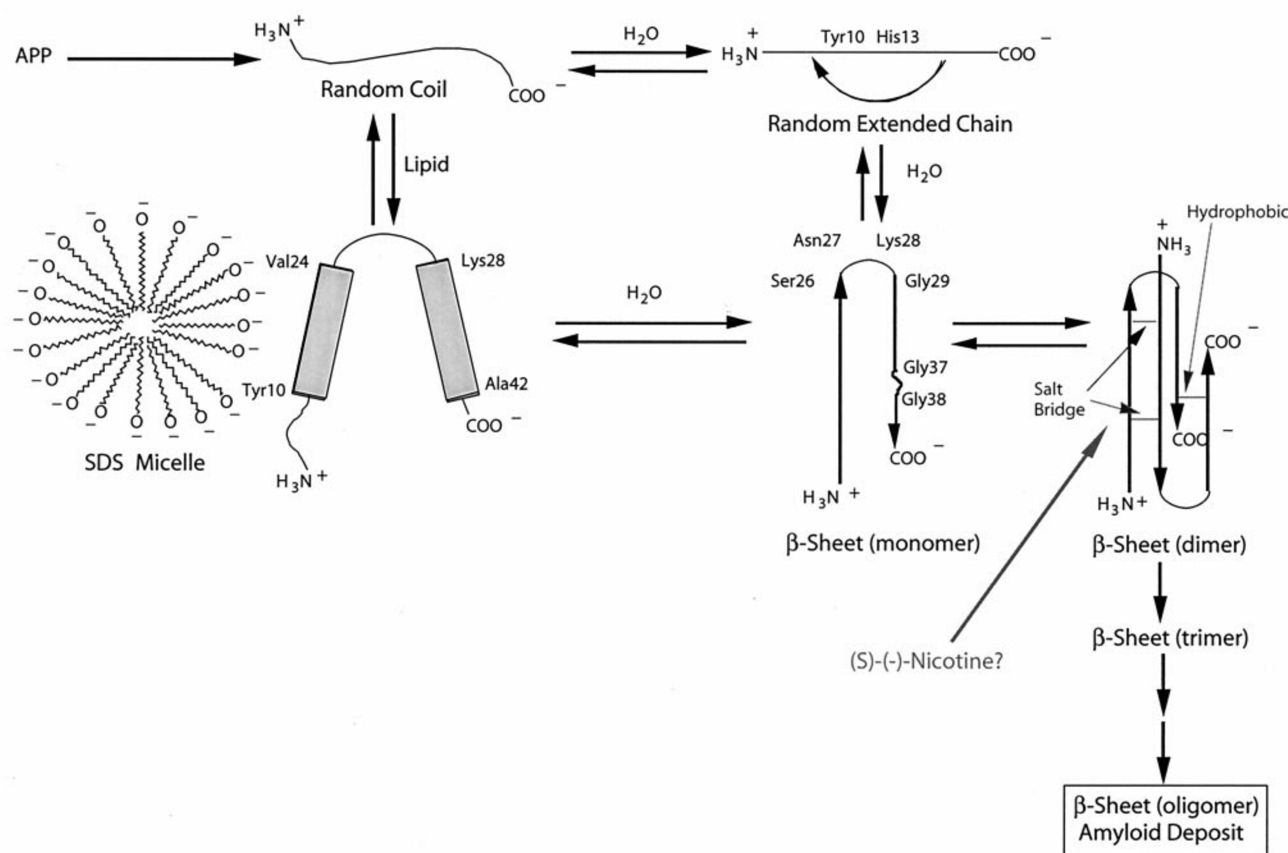


Figure 5. Presumed mechanism of β-amyloidosis for the β-amyloid (Aβ) (1–42), which involves formation of a largely monomeric α-helical structure when bound to lipids (Shao et al 1999) and a predominantly monomeric random, extended chain structure in water solution. The α-helices are depicted with darkened cylinders, and the random coil or extended strand regions are drawn with wavy lines. The initially formed monomeric β-sheet structure is presumptive and is based on previous studies showing a reverse turn at Ser26–Asn27–Lys28–Gly29 (Hilbich et al 1991; Lee et al 1995). In the brain, after proteolytic cleavage of the amyloid precursor protein (APP), the Aβ(1–42) presumably monomeric structures that can aggregate into β-sheet–like structures as a result of possible brain microenvironmental changes such as localized regions of high peptide concentration or possibly small amounts of preformed β-sheet ("seed") material. The α-helical structure remains on the sodium dodecyl sulfate (SDS) or lipid surface and does not become imbedded into the hydrophobic interior. On the basis of the present nuclear magnetic resonance data, nicotine does not bind to the α-helical or random extended chain structures, suggesting that the inhibition may result from binding to a small β-sheet aggregate. A more effective nicotinelike amyloid inhibitor could be designed to bind to either the random, extended chain structure or α-helical structure, thereby slowing down aggregation to the toxic β-sheet structures that eventually produce amyloid.

strated that nicotine binds to the His side-chains when folded in an α-helical conformation (Salomon et al 1996; Zagorski 1999). Figure 2 shows the upfield spectral regions in SDS solution of the Aβ(1–42) alone (0.5 mmol/L) (Figure 2A), nicotine alone (Figure 2E), and the Aβ(1–42) with varying amounts of nicotine (0.25 mmol/L [Figure 2B], 0.50 mmol/L [Figure 2C], and 1.0 mmol/L [Figure 2D]). The observed chemical shift changes of nicotine when mixed with the Aβ(1–42) are summarized in Table 1. The signals showing the most pronounced changes in chemical shifts are for the 2′H, 5′CH_2, and N-CH_3 of nicotine, which are significantly less in magnitude than in previous NMR studies of the Aβ(1–28) (Salomon et al 1996). The binding is in fairly rapid exchange, as shown by single averaged NMR peaks and the lack of any linearity between the chemical shift and nicotine concentration. Most of the signals show upfield chemical shift changes, and the largest shifts occur in the 5-membered *N*-methylpyrrolidine ring.

To further explore the possibility of binding, we obtained two-dimensional HSQC NMR data with uniformly ^{15}N-labeled Aβ(1–42) in SDS solution. The HSQC contains cross-peaks for all protonated nitrogens (^{15}N-H bonds) and thus provides a fingerprint map of a protein. The binding of a ligand induces movement of specific cross-peaks indicative of particular binding locations in the protein. Recently, the HSQC experiment has emerged as a powerful tool for the rapid screening of compounds

Table 1. Chemical Shifts in the Complex of Nicotine and the 42-Residue β-Amyloid Peptide in Sodium Dodecyl Sulfate Solution

NMR resonance of nicotine	Bound chemical shift (ppm)[a]
2H	−0.03
6H	−0.01
4H	0.00
5H	0.01
2H′	−0.10
$5CH_2'$	−0.04
	−0.07
$3CH_2'$	0.00
$4CH_2'$	−0.01
$N\text{-}CH_3$	−0.05

NMR, nuclear magnetic resonance.
[a] Obtained by subtracting the chemical shifts in nicotine from those seen in the complex. The negative shifts are upfield.

that can facilitate the design of more specific drug candidates (Hajduk et al 1999; Shuker et al 1996). When compared to conventional assays, the high-throughput HSQC-based screening approach can identify high-affinity ligands for protein targets with no known function.

The HSQC spectra were obtained in SDS solution at neutral pH with the Aβ(1–42) at 0.25 mM concentration. Incremental titrations of (*R*)-(+)-nicotine (the inactive isomer) and (*S*)-(−)-nicotine (the active isomer) were added to separate peptide solutions, in which the peptide concentration was constant (0.25 mM) and the nicotine concentration varied (0.050 mM, 0.15 mM, 0.25 mM, and 0.50 mM). The HSQC data were acquired within 10 min after each addition of the nicotine, and the pH was checked to ensure adequate buffering capacity. The NMR data showed no changes in chemical shifts, thus establishing that (*R*)-(+)-nicotine and (*S*)-(−)-nicotine do not bind to the α-helical structure of the Aβ(1–42) in a manner that induces changes in the backbone secondary structure.

NMR Studies with the Random, Extended Chain Structure

We have recently completely assigned the ^{1}H and ^{15}N NMR assignments of the Aβ(1–42) in water solution at pH 7.2 (Shao et al, submitted). Overall, the NMR data demonstrate that the peptide adopts a predominantly random, extended chainlike structure. These results suggest that by itself the Aβ(1–42) peptide may be soluble and that brain microenvironmental changes in the AD brain may be required to induce a random, extended chain→β-sheet conversion.

To analyze whether or not nicotine binds to the random, extended chainlike structure, we obtained two-dimensional HSQC data NMR with uniformly ^{15}N-labeled peptide, in an analogous manner as done in the SDS solution. The HSQC spectra were obtained at neutral pH with gradual titration of (*R*)-(+)-nicotine (the inactive isomer) and (*S*)-(−)-nicotine (the active isomer) to separate Aβ(1–42) solutions; the peptide concentration was maintained constant (0.20 mM), whereas the nicotine concentration varied (0.050 mM, 0.15 mM, 0.25 mM, and 0.50 mM). A representative HSQC is shown in Figure 3 for the Aβ(1–42) alone and with 3 molar equivalents of (*S*)-(−)-cotinine, (*R*)-(+)-nicotine, and (*S*)-(−)-nicotine. Cotinine is the major metabolite of nicotine and is readily excreted in the urine (Benowitz 1996). The HSQC spectra show a narrow NH chemical shift dispersion, in support of a predominantly random, extended chain structure. The spectra for 0.40 mM of (*S*)-(−)-cotinine and (*R*)-(+)-nicotine were identical to that of the Aβ(1–42) alone, without any major chemical shift changes. These data suggest that (*S*)-(−)-cotinine and (*R*)-(+)-nicotine do not bind to monomeric Aβ(1–42) when folded as a random, extended chain structure. Still, the spectra for Aβ(1–42) with (*S*)-(−)-nicotine showed minor perturbations, and expanded plots are shown in Figure 4. These spectra indicate that (*S*)-(−)-nicotine induces slight chemical shift variations of the cross-peaks for Tyr10, Glu11, Val12, and His13, which includes weakening of the signal intensities and a doubling of the Val12 peak. However, the magnitude of these variations and the lack of any concentration-dependent chemical shift changes suggest that (*R*)-(+)-nicotine, (*S*)-(−)-nicotine, and (*S*)-(−)-cotinine do not bind to the random extended chain structure of the Aβ(1–42).

Discussion

The molecular mechanisms for the accumulation of the Aβ peptide into insoluble amyloid remain largely unknown. A highly regarded mechanism involves an "amyloid-initiated cascade" pathway, where altered production, removal, and aggregation of the Aβ peptide initiate a sequence of events that leads to neuronal death (Selkoe 1999). Besides amyloid, other lesions such as the neurofibrillary tangles are also abundant in AD brains, and it may happen that the tangles are more important in the pathogenesis of the disease. The tangles are intracellular deposits consisting of twisted filaments of the cytoskeletal Tau protein. However, since the majority of genetic and biological data support a critical role for amyloid formation in AD, the amyloid-initiated cascade hypothesis appears to be the most promising model for drug discovery.

An intense area of research in AD involves identifying potential inhibitors that either slow down or prevent the precipitation of the β-peptide into amyloid. Numerous studies indicate that Aβ amyloid inhibition is a good

therapeutic approach for treatment of AD. Because monomeric and potentially toxic, proteolytic-resistant (Garzon-Rodriguez et al 1997; Roher et al 1996), oligomeric species of Aβ exist in equilibrium within tissue culture medium (Esler et al 1996; Huang et al 1997; Johnstone et al 1996; Knauer et al 1992; LeVine 1995; Pike et al 1991), the Aβ monomer is perhaps the best therapeutic target for binding by an amyloid-formation inhibitor. On the basis of reports of an inverse relationship between the risk of AD and cigarette smoking (Friedland 1994; Lerner et al 1997), we have been studying the effects of nicotine and its metabolite, cotinine, on the solution conformations and aggregational properties of the β-peptide (Salomon et al 1996; Zagorski 1999).

A possible mechanism for β-amyloidosis once the β-peptide is released from the APP is outlined in Figure 5. The various pathways shown connect the in vitro biophysical studies to a natural situation that may exist in the brain. The model emphasizes a conformationally driven mechanism in which the three major solution structures of the β-peptide coexist in equilibrium: random coil (monomeric, water solution), α-helix (monomeric, in membranelike conditions similar to the SDS micelle), and the β-sheet (oligomeric). The aggregated β-sheet is the predominant structural motif in the amyloid plaques. When the aggregation reaches a critical mass, the β-sheet structure precipitates as amyloid, and reconversion back to soluble random coil or α-helical structures is no longer possible.

From the NMR data, we can conclude that nicotine does not show any significant binding to either the α-helical or the random extended chain structures. Thus, the nicotine inhibition to amyloid formation may result from binding to a small-sized soluble β-sheet aggregate, which is not yet detectable by NMR methods. Further studies with the Aβ(1–42) β-sheet structure as well as with additional nicotine analogs are required to support this binding mode. Potential analogs could be synthesized with electron-donating substituents in the 4 position of the pyridine ring of nicotine, since this would increase the basicity of the N-1 nitrogen and perhaps increase binding. An analogous structural motif may be involved in the binding of the Aβ peptide to transthyretin, a normal protein component of plasma (Schwarzman et al 1994). More recent work established that the Aβ(1–42) binds to the α7 nicotinic acetylcholine receptor site, and the critical epitope is located with the Val12–Lys28 region (Wang et al 2000). Although it is not yet known whether nicotine does indeed bind to the Aβ in the human brain, the possibility of such an event is intriguing. We anticipate that additional studies (directed at monomeric Aβ) may facilitate the development of more selective nicotinelike inhibitors to prevent amyloid plaque formation in AD.

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