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Aβ40 Protects Non-toxic Aβ42 Monomer from Aggregation

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Aβ40 and Aβ42 are the predominant Aβ species in the human body. Toxic Aβ42 oligomers and fibrils are believed to play a key role in causing Alzheimer's disease (AD). However, the role of Aβ40 in AD pathogenesis is not well established. Emerging evidence indicates a protective role for Aβ40 in AD pathogenesis. Although Aβ40 is known to inhibit Aβ42 fibril formation, it is not clear whether the inhibition acts on the non-toxic monomer or acts on the toxic Aβ42 oligomers. In contrast to conventional methods that detect the appearance of fibrils, in our study Aβ42 aggregation was monitored by the decreasing NMR signals from Aβ42 monomers. In addition, differential NMR isotope labelling enabled the selective observation of Aβ42 aggregation in a mixture of Aβ42 and Aβ40. We found Aβ40 monomers inhibit the aggregation of non-toxic Aβ42 monomers, in an Aβ42/Aβ40 ratio-dependent manner. NMR titration revealed that Aβ40 monomers bind to Aβ42 aggregates with higher affinity than Aβ42 monomers. Aβ40 can also release Aβ42 monomers from Aβ42 aggregates. Thus, Aβ40 likely protects Aβ42 monomers by competing for the binding sites on pre-existing Aβ42 aggregates. Combining our data with growing evidence from transgenic mice and human genetics, we propose that Aβ40 plays a critical, protective role in Alzheimer's by inhibiting the aggregation of Aβ42 monomer. Aβ40 itself, a peptide already present in the human body, may therefore be useful for AD prevention and therapy.

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Keywords: Alzheimer's disease; Aβ40; Aβ42; NMR; aggregation

Alzheimer's disease (AD) is the most common type of senile dementia, but its etiology and pathogenesis remain poorly understood.[1] AD pathology is characterized by extracellular senile plaques and intracellular neurofibrillary tangles in affected brains. Amyloid β-peptides (Aβ) are the major components of the senile plaques, generated from the sequential cleavage of the amyloid precursor protein (APP) by the β and γ-secretases. A small percentage of AD cases are hereditary and are known as familial Alzheimer's disease (FAD). The critical role of Aβ in AD has been underscored by the fact that FAD mutations occur only in genes involved directly in Aβ production: APP, presenilin 1 (PS1) and presenilin 2 (PS2) (the active site residues of γ-secretase are in PS1 and PS2). Aβ peptides range mostly from 39 to 43 residues in length. Aβ40 and Aβ42, composed of 40 and 42 amino acid residues, respectively, constitute the bulk of Aβ species in plasma, cerebrospinal fluid and plaques.[2] In the popular amyloid cascade hypothesis for AD pathogenesis, toxic Aβ42 aggregates such as Aβ42 oligomers and fibrils play a dominant role in causing AD.[3,4] This is supported by the increased ratio of Aβ42/Aβ40 with many FAD mutations,[5–8] the enhanced aggregation of Aβ42,[9–11] and neurotoxicity of Aβ42 aggregates.[12–23] Controversy exists concerning the roles of Aβ42 fibrils and Aβ42 oligomers in causing AD. Aβ42 fibrils[12,16,17] or protofibrils[18] are toxic to neurons. However, there is evidence showing a correlation between the amount of soluble Aβ oligomer and dementia in AD patients.[24,25] Transgenic mouse models of AD

Abbreviations used: AD, Alzheimer's disease; Aβ, amyloid β-peptide; APP, amyloid precursor protein; FAD, familial Alzheimer's disease; PS1, presenilin 1; PS2, presenilin 2; HSQC, heteronuclear single quantum coherence.

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show functional changes before the appearance of plaques.[26,27] Recently, the neurotoxic effects of Aβ42 soluble oligomers have been characterized extensively.[13–15,19,28,29] Many forms of the Aβ42 oligomer, such as dimer,[21] trimer[20] and 12mer,[14,22] were shown to be toxic but the actual Aβ42 oligomers that play a causative role in AD have not been defined.[14,19,20,22,23] In contrast, the Aβ42 monomer is not toxic.[29–31] Thus, any agent that keeps Aβ42 monomers from aggregation can have a protective role against the development of AD by reducing the generation of toxic Aβ42 species.

The role for Aβ40 in AD pathogenesis has not been well-established. Recently, several studies have suggested a protective role for Aβ40 in AD. There was no plaque formation in transgenic mice overexpressing Aβ40.[32] Amyloid deposition was reduced dramatically by crossing Aβ40 over-expressing mice with AD mouse model Tg2576 or Aβ42 over-expressing transgenic mice.[33] Exacerbated plaque pathology was observed in transgenic mice with selectively reduced levels of Aβ40.[34] Reduced levels of Aβ40 were found with numerous FAD mutations, and were correlated with accelerated onset of dementia.[35,36] However, it is not clear how Aβ40 executes its protective function in AD on a molecular level. It has been shown that Aβ40 inhibits Aβ42 fibril formation.[37–39] There can be two very different scenarios for Aβ40 inhibition of Aβ42 fibril formation. The inhibition may act on the non-toxic monomer or act on the toxic Aβ42 oligomers. Thus, inhibition may either protect the benign Aβ42 monomers from aggregation or it may trap Aβ42 in oligomeric forms that may be highly toxic. Whether Aβ40 can inhibit toxic Aβ42 oligomer formation and, more importantly, whether Aβ40 can keep Aβ42 in a non-toxic, monomeric state has not been studied. In this solution NMR study, we showed that Aβ40 monomers protect non-toxic Aβ42 monomers against aggregation in a Aβ42/Aβ40 ratio-dependent manner and we explored the mechanism and significance of the protective effect of Aβ40.

Aβ40 specifically inhibits the aggregation of non-toxic Aβ42 monomers

To probe whether Aβ40 monomers can inhibit the aggregation of Aβ42 monomers, we developed an Aβ42 monomer stability and aggregation assay using solution NMR. NMR is the ideal tool for tracking the aggregation of specific Aβ species in a mixture of Aβ (e.g. Aβ40 and Aβ42) by selective isotope labelling. ^{1}H-^{15}N heteronuclear single quantum coherence (HSQC) was used to selectively monitor the aggregation of ^{15}N labelled Aβ42 monomers in the presence of unlabeled Aβ40 monomers. The HSQC experiments were exclusively detecting NMR signals from ^{15}N-labeled Aβ42 monomers prepared by treatment with NaOH as established previously at low temperatures.[40,41] In addition, the monomeric state of Aβ42 detected by NMR in our aggregation experiments was demonstrated by translational diffusion coefficient between 0 °C and 37 °C, and by glutaldehyde crossing-linking experiments (Supplementary Data Figure 1). The aggregation of Aβ42 was monitored by the decrease of HSQC signal of Aβ42 monomers as the Aβ42 monomers aggregate into NMR-invisible, high molecular weight fibrils. Aβ42 fibril formation was confirmed by thioflavin T assay and atomic force microscopy (Supplementary Data figure 2). NMR signals from Aβ42 monomers decreased quickly in 3 h at 25 °C (Figure 1(a) and (b)). Strikingly, signals from Aβ42 monomers did not decrease in the presence of four molar equivalent of Aβ40 monomers at 25 °C over 3 h, indicating that Aβ40 keeps Aβ42 in a monomeric state and inhibits the aggregation of Aβ42 (Figure 1(c) and (d)). Native PAGE and Western blot were employed to further confirm the anti-Aβ42 aggregation effect of Aβ40. The detection of Aβ42 signal in the presence of Aβ40 was achieved by an Aβ42-specific antibody in a Western blot of the native protein gel. As shown in Figure 1(e), without Aβ40, the band representing Aβ42 monomers decreased quickly in intensity at 25 °C and disappeared overnight; while in the presence of four molar equivalent of Aβ40 monomers, there was little change in the intensity of the Aβ42 monomer band even after incubation overnight at 25 °C (Figure 1(f)). It has been shown that Aβ40 inhibits Aβ42 fibril formation by monitoring the appearance of fibrils[37–39] but it is not clear whether the inhibition protects the benign Aβ42 monomers from aggregation or the inhibition traps Aβ42 in toxic oligomeric forms. If Aβ40 inhibition blocks Aβ42 fibril formation at the oligomer stage, Aβ40 would not have inhibited the decrease of the signal from Aβ42 monomers during Aβ42 aggregation. In addition, if significant amount of low molecular weight Aβ42 oligomers (e.g. dimers or trimers) were formed by Aβ40 inhibition, such species would have been detected by NMR. However, NMR signals from low molecular mass Aβ42 oligomers were never observed in our aggregation experiments. Thus, our NMR results demonstrated that Aβ40 inhibition of Aβ42 fibril formation acts on Aβ42 monomers and protects Aβ42 in the non-toxic, monomeric form instead of trapping Aβ40 in oligomeric forms.

A series of control experiments were carried out to ensure that Aβ40 indeed specifically inhibits the aggregation of Aβ42 monomers (Figure 2(a)). We used both recombinant (rPeptide†) and synthetic Aβ40 peptides from two companies (Bachem and BioSource) to demonstrate that the anti-aggregation effect did not originate from particular preparations or sources of Aβ40 (Figure 2(a)). We again used both recombinant (rPeptide) and synthetic Aβ42 peptides (Bachem and BioSource) to demonstrate by Western blot of native gels (data not shown) that Aβ40 inhibited the aggregation of Aβ42 peptides from all three different sources. Negative controls were carried out with ubiquitin, BSA and sequence-reversed Aβ(40-1), none of which had any anti-

† http://www.rpeptide.com

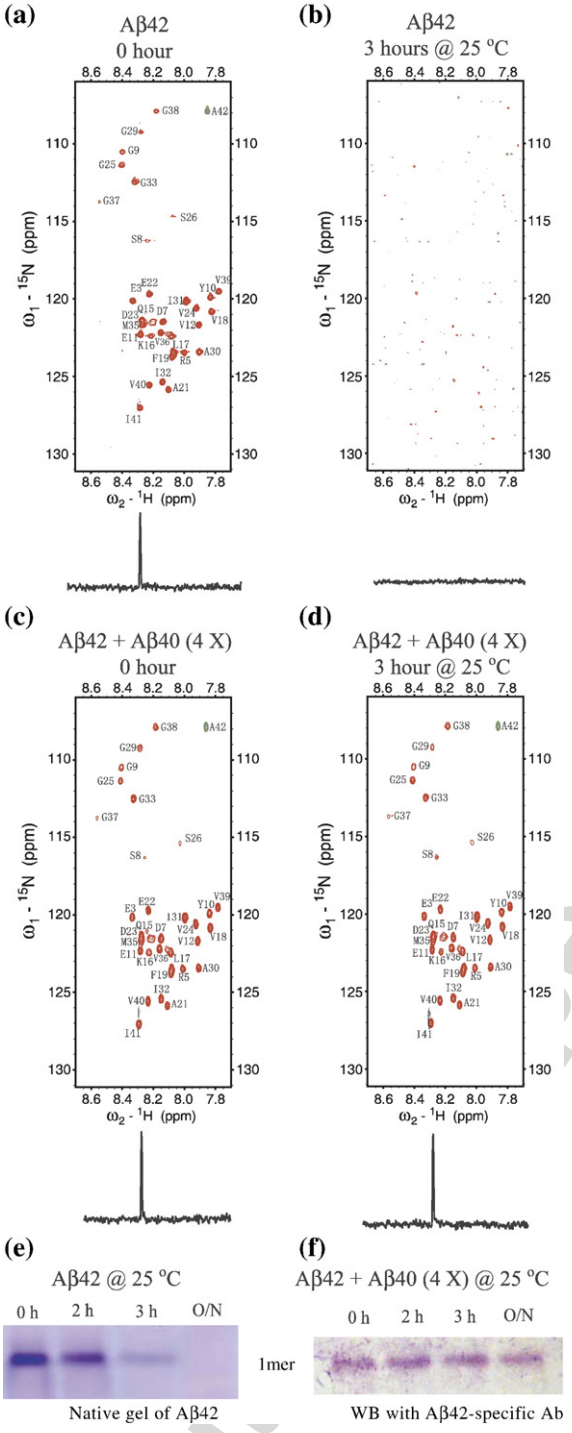

Figure 1. Aβ40 protects Aβ42 monomers from aggregation. (a) and (b) The 2D ^{15}N-^{1}H HSQC spectra of Aβ42 NMR samples at 0 and 3 h at 25 °C, respectively, with the 1D proton trace at I41 ^{15}N frequency presented below the 2D HSQC. After 3 h at 25 °C, almost all Aβ42 monomer signals disappeared. (c) and (d) The 2D ^{15}N-^{1}H HSQC spectra of Aβ42 NMR samples in the presence of 4X Aβ40 monomers at time zero and after 3 h at 25 °C, respectively, with the 1D proton trace at I41 ^{15}N frequency presented below the 2D HSQC. In contrast to Aβ42 alone, in the presence of four molar equivalents of Aβ40, the Aβ42 monomer signal remained intact after 3 h of incubation at 25 °C. (e) Native gel of Aβ42 with samples taken from different time-points at 25 °C. The intensity of Aβ42 monomer bands decreased rapidly in the absence of Aβ40. (f) Western blot of the native gel of Aβ42 in the presence of four molar equivalents of Aβ40 at various time-points at 25 °C. The Aβ42 monomer bands were stable even after incubation overnight at 25 °C, demonstrating the protective effect of Aβ40 against the aggregation of Aβ42 monomers. The Western blot was performed with Aβ42 from rPeptide, Bachem and BioSource and similar results were obtained. The Aβ42 NMR sample concentrations were 20 μM. Aβ40 and Aβ42 peptides were obtained from rPeptide (http://www.rpeptide.com; catalogue numbers A-1101-1, A-1102-1, A-1001-2 and A-1002-2 for ^{15}N labelled Aβ40, ^{15}N labelled Aβ42, natural abundance Aβ40 and natural abundance Aβ42, respectively.), Bachem (catalogue numbers H-1194, H-1368 and H-2972 for Aβ40, Aβ42 and sequence reversed Aβ(40-1) and BioSource (catalogue numbers 03136 and 03111 for Aβ40 and Aβ42). The Aβ monomers were prepared using the sodium hydroxide method as described.[41,48] The lyophilized Aβ powder obtained from commercial sources was dissolved in 10 mM NaOH to 1 mg/ml and then sonicated for 1 min in a waterbath to disaggregate Aβ. Natural abundance Aβ40 and ^{15}N-labeled Aβ42 were mixed to desired ratios. Aβ was then diluted to the desired concentration using 20 mM potassium phosphate buffer and the pH was adjusted to 7.2 using TFA. A control ^{15}N Aβ42 sample was always prepared by adding the same amount of NaOH and adjusting the sample pH to 7.2 using TFA. The 2D ^{15}N-^{1}H NMR HSQC spectra of the freshly prepared ^{15}N Aβ42 samples with different molar ratios of Aβ40 were collected consecutively at the desired temperature (25 °C or 37 °C in this study) on an 800 MHz Bruker Avance NMR spectrometer equipped with a cryoprobe. The ^{15}N-^{1}H HSQC and the 1D ^{15}N-selected experiments detected signals only from Aβ monomers.[40] The recycle delay was 1.5 s, and the number of scans was 8. For 2D ^{15}N-^{1}H HSQC, 64 complex points were taken in the ^{15}N dimension and 1024 complex points were taken in the ^{1}H dimension. The total acquisition time was ~30 min. The intensity of the strongest peak (A42) in Aβ42 was used as an indication of relative Aβ42 monomer concentration. When the aggregation was too fast to be monitored by 2D NMR, ^{15}N-selected 1D was used instead. In 1 D NMR spectra, areas under the peaks were integrated between 7.8 ppm and 8.5 ppm as an indication of relative monomer concentration. The sample temperature was carefully calibrated using 100% methanol. For ^{15}N-selected 1D, the number of scans was 256 with total acquisition time of ~7 min. The data were processed with Topspin (Bruker Biospin), NMRPipe and Sparky (http://www.cgl.ucsf.edu/home/sparky/). For native gel analysis of Aβ aggregation, Aβ42 samples incubated with or without four molar equivalents of Aβ40 were analyzed by native PAGE (15% (w/v) polyacrylamide gel; BioRad catalogue number 161–1103). WesternBreeze Chromogenic Immunodetection Kit (Invitrogen catalogue number WB7105) was used for Western blots and Aβ42 specific antibody (Chemicon catalogue number AB5739, 1:5000 dilution) was used as the primary antibody.[29] Aβ42 antibody did not recognize Aβ40 in the native gel (data not shown).

aggregation effect upon Aβ42 (Figure 2(a)). Thus, the inhibition of Aβ42 monomers aggregation is specific to Aβ40.

M35 oxidation is not a factor in our aggregation experiments. Aβ42 and Aβ40 purchased from

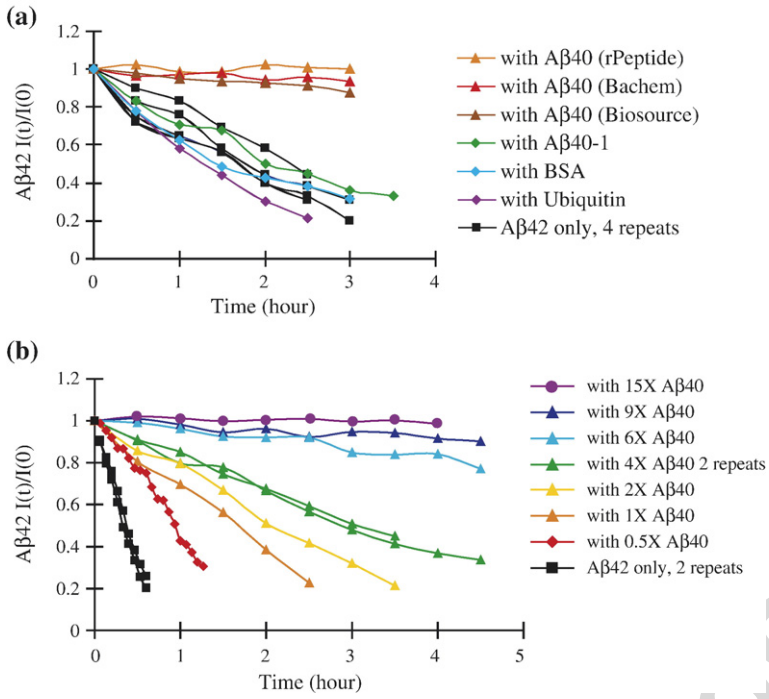

Figure 2. Aβ40 inhibits the aggregation of Aβ42 monomers in a specific and Aβ42/Aβ40 ratio-dependent manner. (a) Aβ42 monomer aggregation assay with different sources of Aβ40 and control proteins; 20 μM ^{15}N-labelled Aβ42 monomers mixed with various unlabeled peptides or proteins at a concentration of 80 μM. $I(t)/I(0)$ is the ratio of the NMR signal intensity at time t over the NMR signal intensity at time zero. (b) The effect of Aβ42/Aβ40 ratio on the aggregation of Aβ42 monomers detected by NMR. The Aβ42 NMR sample concentrations were 20 μM. Aβ40 monomers were added to the Aβ42 NMR samples to different end concentrations (0, 10 μM, 20 μM, 40 μM, 80 μM, 120 μM, and 180 μM) to achieve Aβ40/Aβ42 ratios of 0, 0.5, 1, 2, 4, 6 and 9, respectively. Increasing the ratio of Aβ40/Aβ42 stabilized Aβ42 monomers (shown by $I(t)/I(0)$), indicating that greater amounts of Aβ40 prevent the aggregation of Aβ42 monomers more efficiently.

rPeptide are in the M35 reduced state, as shown by mass spectrometry. We obtained a molecular mass of 4567.7 Da, corresponding to 98% ^{15}N labelled Met35 reduced Aβ42. M35 oxidation of the NMR Aβ42 sample would have resulted in an increase in molecular mass of 16 Da. In addition, ^{15}N-^{1}H HSQC spectra of M35-reduced and M35-oxidized Aβ have distinct chemical shifts for M35 and residues surrounding M35.[41] Both HSQCs of Aβ42 and Aβ40 used in this study gave rise to spectral pattern corresponding to M35-reduced Aβ. The spectral pattern did not change during the course of the aggregation experiment, indicating M35 stays reduced in our experimental conditions. We have re-dissolved Aβ42 aggregates using DMSO after the NMR aggregation experiment and showed by MS that Aβ42 was still in the Met35-reduced form.

Aβ40 protects Aβ42 monomers in an Aβ42/Aβ40 ratio-dependent manner

Since different Aβ42/Aβ40 ratios exist in physiological and pathological conditions (e.g. in FAD),[42,43] we investigated how different Aβ42/Aβ40 ratios affect the aggregation of Aβ42 monomers. As the initial Aβ40/Aβ42 monomer ratio increased from 0 to 15, the monomer Aβ42 signal decreased progressively more slowly over time at the physiological temperature of 37 °C (Figure 2(b)). Thus, the anti-Aβ42 aggregation effect of Aβ40 was Aβ42/Aβ40 ratio-dependent and became stronger with a lower ratio of Aβ42/Aβ40. In contrast, increased Aβ42/Aβ40 ratios will result in increased Aβ42 aggregation. The results in Figure 2(b) clearly explain how an increased Aβ42/Aβ40 ratio may eventually lead to dementia in FAD by the increased accumulation of toxic Aβ42 aggregates. In a number of the FAD mutations, e.g. APP V715M, PS1-Δ9 and PS1-L166P, the level of Aβ42 is not increased significantly; in contrast, the level of Aβ40 is decreased.[35,36,44,45] If Aβ42 played a dominant role in causing FAD, an increased level of Aβ42 alone, instead of the increased Aβ42/Aβ40 ratio, should have been observed with all FAD mutations. This is a subtle yet important contradiction to the current amyloid hypothesis, suggesting equally important roles for Aβ40 and Aβ42 in AD pathogenesis. In FAD cases with reduced levels of Aβ40, restoring normal levels of Aβ40 and the Aβ42/Aβ40 ratio may result in better inhibition of the formation of Aβ42 aggregates, indicating Aβ40 as a novel therapy for the prevention and treatment of FAD. Because a lower than the usual Aβ42/Aβ40 ratio (1:15 compared with 1:9) still provided better protection against Aβ42 aggregation (Figure 2(b)), increasing the levels of Aβ40 may have enhanced inhibition on the formation of Aβ42 toxic aggregates in AD patients. Thus Aβ40 might be useful for preventing AD as well.

Aβ40 inhibits Aβ42 aggregation by competitively binding to Aβ42 aggregates

We next explored the mechanisms of Aβ40 inhibition of Aβ42 aggregation. The fast aggregation of Aβ occurs mostly through the binding of Aβ monomers with pre-existing aggregates or seeds. We tested the hypothesis that Aβ40 monomers might preferentially bind to the Aβ42 aggregates and block the binding site of Aβ42 monomers on

Aβ42 aggregates. Aβ42 aggregates were generated by incubating NaOH-treated Aβ42 at 37 °C for 1 h, after which all monomer NMR signals disappeared with concomitant Aβ42 fibril formation. When Aβ42 aggregates were titrated into Aβ monomer solutions and bound to monomers, the decrease in Aβ monomer signal reflected the binding affinity between Aβ monomer and Aβ42 aggregates. As shown in Figure 3(a), in the presence of the same amount of Aβ42 aggregates, the decrease of Aβ40 monomer signal was always more pronounced than that of the Aβ42 monomer, suggesting that Aβ40 monomers bind to the Aβ42 aggregates with a higher affinity than Aβ42 monomers.

We further explored the possibility that Aβ40 monomers might replace Aβ42 molecules on the Aβ42 aggregates and release Aβ42 monomers. Aβ40 monomers were added to an aggregated sample of [^{15}N]Aβ42. With the addition of Aβ40 monomers, Aβ42 monomer signals reappeared, while the reverse Aβ40-1 sequence had no such aggregate-dissolution effect (Figure 3(b)). This aggregate-dissolution effect was Aβ40 monomer concentration-dependent, as shown in Figure 3(c).

We propose two mechanisms for the anti-Aβ42 aggregation effect of Aβ40. First, Aβ40 binds to the aggregates of Aβ42, blocking the binding site for Aβ42 monomer and thereby inhibiting the aggregation of Aβ42 monomers (Figure 4(a)). Second, Aβ40 monomers can replace Aβ42 on Aβ42 aggregates, releasing Aβ42 monomers from the aggregates (Figure 4(b)).

Revision of the current amyloid hypothesis and implications for AD prevention and treatment

We propose a modification of the current amyloid hypothesis as shown in Figure 4(c);[4] that is, Aβ40 plays an opposite but equally important role as Aβ42 in the pathogenesis of AD. This modification is based on several lines of evidence: (1) Aβ40 protects Aβ42 in the monomeric, non-toxic state by competitively binding to Aβ42 aggregates. (2) Aβ40 inhibits Aβ42 fibril formation *in vitro*[37–39] and *in vivo*.[33] (3)Aβ40 protects neurons from Aβ42 toxicity in neuronal cultures and in rat brain.[39,46] (4) A lowered level of Aβ40 leads to exacerbated plaque pathology in APP-transgenic mice.[34] (5) In human genetics, it is the Aβ42/Aβ40 ratio, not the level of Aβ42 alone that correlates with FAD, suggesting a pivotal, protective role for Aβ40. (6) With many FAD mutations, the level of Aβ40 is decreased while the level of Aβ42 does not change.[35,36,47] (7) A decreased level of Aβ40 alone correlates significantly with the accelerated onset in FAD.[36] Thus, Aβ40 is likely a built-in defence mechanism against Aβ42 aggregation and the generation of toxic Aβ42 oligomers and fibrils. When this defence mechanism is weakened by FAD mutations, more neurotoxic Aβ42 species are formed, initiating and accelerating the development of AD. Thus, the balance of Aβ40 and Aβ42 is crucial for the normal brain. The

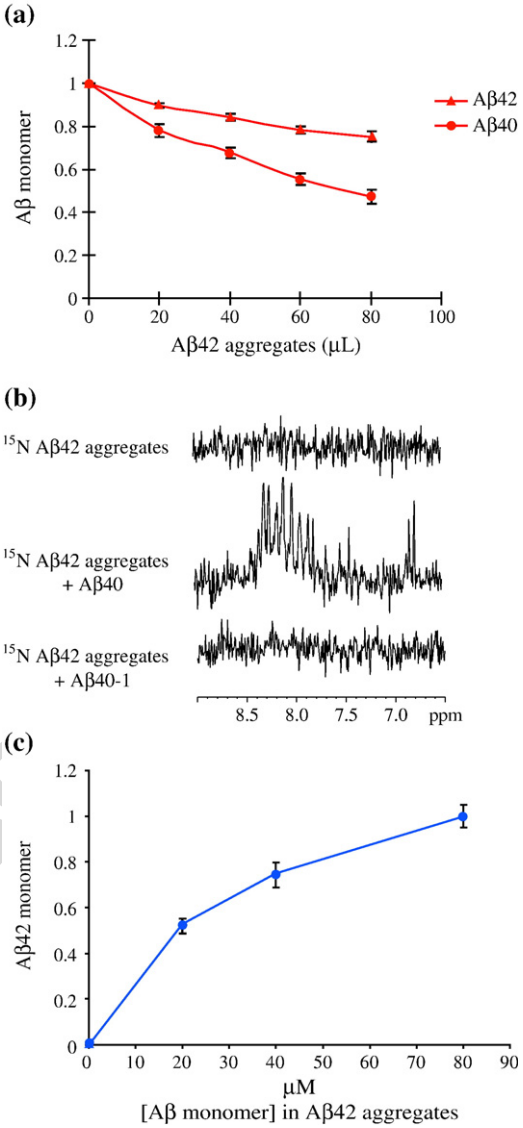

Figure 3. Interactions between Aβ40 and Aβ42 in Aβ42 aggregation. (a) Titration of Aβ42 aggregates into Aβ monomers. Aβ42 aggregates were generated by incubating NaOH-treated Aβ42 at 37 °C for 1 h, after which all Aβ42 monomer NMR signals disappeared. Aβ42 aggregates were titrated into ^{15}N-labelled Aβ40 and ^{15}N-labelled Aβ42 monomers separately. Aβ40 monomer signals (●) decreased faster than Aβ42 monomer signals (▲) with the addition of Aβ42 aggregates, suggesting that Aβ40 binds to Aβ42 aggregates with higher affinity. (b) Aβ40 monomers released Aβ42 monomers from Aβ42 aggregates. No signal was observed from an ^{15}N-selected 1D NMR spectrum of ^{15}N-Aβ42 aggregates. However, with the addition of four molar equivalents of unlabelled Aβ40 monomers, ^{15}N-Aβ42 monomer signal reappeared; while the addition of equal amount of the reverse sequence Aβ40-1 had no such effect. (c) Aβ40 monomers released Aβ42 monomers from aggregates in a concentration-dependent manner. For detecting Aβ42 monomers released from Aβ42 aggregates by Aβ40 monomers, the 1D NMR experiment used 2048 scans with a total acquisition of ~60 min. Error bars were from repeated experiments.

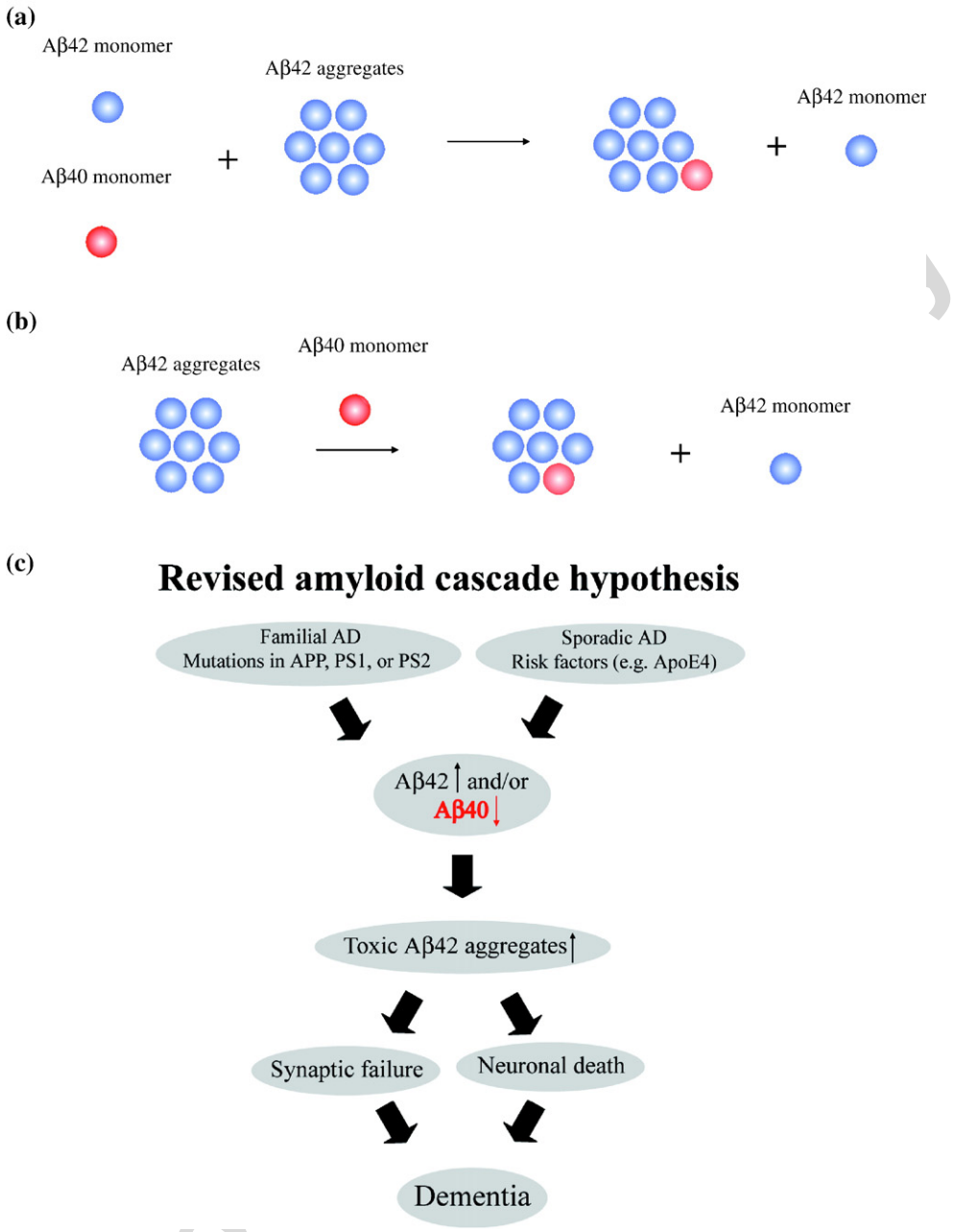

Figure 4. Proposed molecular mechanisms of Aβ40 and the revision of the amyloid hypothesis. (a) Aβ40 competitively binds to Aβ42 aggregates, blocking the binding site for Aβ42 monomers and inhibiting the aggregation of Aβ42 monomers. (b) Aβ40 monomers can compete Aβ42 off Aβ42 aggregates, releasing Aβ42 monomers. (c) The current amyloid hypothesis emphasizes the role of Aβ42 toxicity but neglects the equally important protective role of Aβ40. The amyloid hypothesis is modified to show that decreasing the level of Aβ40 can contribute to the development of Alzheimer's disease. The revision of the amyloid hypothesis immediately suggests Aβ40 itself can be a novel strategy for preventing and treating Alzheimer's disease.

disruption of this balance can lead to AD, as shown by the enhanced ratio of Aβ42/Aβ40 in FAD.

We propose here a novel, testable hypothesis that the peptide Aβ40 might be used for the prevention and treatment of AD. An elegant study by Kumar-Singh *et al*. has established that increased levels of Aβ40 in cell culture transfected with FAD mutants correlates significantly with the increased age of onset of FAD ($p = 0.038$).[36] This supports the hypothesis that enhancing levels of Aβ40 can delay the inevitable onset of dementia in FAD patients. Our results suggest that increasing or restoring the levels of Aβ40 will enhance the stability of non-toxic Aβ42 monomers and therefore may lower the load of neurotoxic species of Aβ42. For young patients with early diagnoses of an FAD-causing mutation, restoring the levels of Aβ40 may be a particularly appealing and promising way to delay or prevent the seemingly inevitable dementia. Due to the similarities between the pathology and clinical

behaviour of FAD and sporadic AD, increasing levels of Aβ40 may have a role in preventing and treating sporadic AD. Aβ40 may have a unique advantage as a disease-modifying strategy for AD, because Aβ40, a peptide already present in the human body, is known to be safe at near-physiological concentrations. Restoring or enhancing the level of Aβ40 may be achieved by supplementing the Aβ40 peptide, modulating γ-secretase specificity and specifically inhibiting the degradation of Aβ40.

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Supplementary Data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jmb.2007.04.014

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