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A Kinetic Model for β -Amyloid Adsorption at the Air/Solution Interface and Its Implication to the β -Amyloid Aggregation Process

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Abstract

The kinetics of adsorption at the air/buffer solution interface of amyloid beta peptide, A β (1–42), whose bulk concentration (submicromolar) is more than two orders of magnitude lower than that typically used in other *in vitro* aggregation studies, has been studied using a Langmuir-Blodgett trough. The pressure–time curves exhibit a lag phase, wherein the surface pressure essentially remains at zero, and a rising phase, corresponding to the A β adsorption at the interface. The duration of the lag phase was found to be highly dependent on both the A β bulk concentration and the solution temperature. A large activation energy (62.2 ± 4.1 KJ/mol) was determined and the apparent adsorption rate constant was found to be linearly dependent on the A β bulk concentration. Attenuated total reflection-IR spectra of the adsorbed A β transferred to a solid substrate and circular dichroism measurements of A β in the solution layer near the interface reveal that the natively unstructured A β in the bulk undergo a conformation change (folding) to mainly the α -helical structure. The results suggest that, prior to the adsorption step, an equilibrium between A β conformations is established within the subsurface. The kinetic equation derived from this model confirms that the overall A β adsorption is kinetically controlled and the apparent rate constant is proportional to the A β bulk concentration. This model also indicates that interfaces such as cell membranes and lipid bilayers may facilitate A β aggregation/fibrillation by providing a thin hydrophobic layer adjacent to the interface for the initial A β conformation change (misfolding) and accumulation. Such a preconcentration effect offers a plausible explanation of the fact that A β fibrillation occurs *in vivo* at nanomolar concentrations. Another important biological implication from our work is that A β misfolding may occur before its adsorption onto a cell membrane. This general kinetic model should also find applications in adsorption studies of other types of biomolecules whose overall kinetics exhibits a lag phase that is dependent on the bulk concentration of the adsorbate.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder underscored by the presence of senile plaques in disease-inflicted brains.^{1, 2} The major components of senile plaques are peptides containing 39–43 amino acid residues (amyloid- β or A β peptides).³ This finding has led to the hypothesis that deposition of A β fibrils and other aggregates is responsible for neuronal cell loss.^{1, 2} However, the causes of A β aggregation and the

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aggregation pathways are still under intense investigation and no unambiguous conclusions have been reached despite the prodigious amount of experimental data.

It is known that in cerebrospinal fluids A β is present at a low concentration (ranging from 0.6 to 8 nM⁴⁻⁹). In an AD-afflicted brain, A β monomers can be converted into insoluble, β -sheet-rich fibrillar plaques and diffusible aggregates from which cytotoxicity has been noted.¹⁰ In contrast, at the concentration commonly found in brains, A β aggregation does not take place *in vitro*.^{11, 12} As a result many *in vitro* aggregation studies have been conducted using A β in the concentration range of 6–40 μ M,¹¹ which is about 3–4 orders of magnitude greater than that found *in vivo*.^{13–15} The validity of *in vitro* studies is justified on the basis that fibrils and other intermediate aggregates formed *in vitro* exhibit the same morphology and structure as those identified in senile plaques. However, a question remaining unanswered is whether A β can aggregate at a much lower concentration *in vitro* and, if it indeed occurs, what factors effect such a process. Evidence has been gathered showing that the cellular milieu either lowers the threshold of A β concentration for the aggregation/fibrillation processes or causes the inhomogeneous distribution of A β near the neuronal membrane. In fact, lipid and cell membranes have been shown to significantly accelerate the A β aggregation^{16–18} through interacting with A β monomers.^{19–22} However, it is not clear whether a heterogeneous interface serves as the nucleation site for A β monomer accumulation or promotes the misfolding (conformation change) of the natively unstructured A β or both. Another unresolved issue is whether the A β conformation change/aggregation occurs prior to its deposition onto cell membranes.

A β peptides are of amphiphilic nature, commonly possessing a hydrophilic domain near the N terminal and a hydrophobic segment close to the C terminal. However, unlike simple surfactants, natively unstructured A β is soluble in aqueous media.^{23–25} Studies have shown that unstructured A β molecules, depending on the specific solution conditions, will transform into α -helical and/or β -sheet conformations at various rates. Though the amphiphilic property of A β has been demonstrated by a few studies^{26–29} and A β is loosely characterized as “surface active” (i.e., having an affinity towards surfaces or interfaces), it is unclear which conformation is surface active and whether conformation change is dependent on the A β monomer concentration.

It has been well documented that outside the cell membrane there exists a solution layer with a thickness of a few nanometers.^{30, 31} A number of physicochemical properties of this layer are different from those of the extracellular matrix. For example, the dielectric permittivity is about one order of magnitude smaller than that of the extracellular matrix and the hydrophobicity is also significantly greater.³¹ Changes in the A β secondary structure and kinetics of structural transformation are shown to be strongly dependent on the hydrophobicity of the environment.^{22, 32–34} Therefore, investigations on how A β adsorbs at an interface and the structural characterization of the resultant aggregates should provide insights into the A β aggregation process and pathogenesis of AD.

The air/solution interface has properties mimicking that between a cell membrane and its extracellular matrix. Therefore, it will be of both fundamental interest and biological relevance to examine the adsorption of A β species at such an interface. Only a few papers focused largely on the interaction of A β with preformed lipid monolayers^{27, 29} and the adsorption of lipid-derivatized A β fragments at the air/water interface.^{35, 36} Moreover, the A β concentration in all of the studies was much greater than the *in vivo* concentration.²⁷ As a consequence, the aforementioned question about whether A β could aggregate at a low level was not addressed. Furthermore, construction of a quantitative kinetic model to explain the A β accumulation at the interface and determination of the activation energy of the A β adsorption has not been carried out. A better understanding of the A β behavior at a

concentration lower than other *in vitro* studies at air/solution interface should shed light onto the role played by the cell membrane. We measured the adsorption kinetics of A β by monitoring the surface pressure change in a Langmuir-Blodgett (LB) trough. We further determined the secondary structure of A β adsorbed onto the interface by attenuated total reflection (ATR)-FTIR and circular dichroism (CD) and examined the morphologies and coverage of the adsorbed layer with atomic force microscopy (AFM). Effects of the interface, A β concentration, and solution temperature on the A β adsorption kinetics are delineated by an equilibration between two different A β conformations prior to the interfacial adsorption step. The model and the derived kinetic rate equation suggest that, though the concentration in cerebrospinal fluid is low, A β undergoes a conformation change before being adsorbed to form a dense layer. Owing to this unusual accumulation/conformation change near the interface, the pressure-time curve displays a lag phase followed by an adsorption step, both of which are dependent on the A β bulk concentration and solution temperature.

Experimental

Material

Lyophilized A β (1–42) was purchased from American Peptide Co. Inc. (Sunnyvale, CA). Other chemicals (Sigma-Aldrich) were of analytical grade. All of the aqueous solutions were prepared using water purified by a Millipore system (Simplicity, Belleria, MA) to a resistivity of 18 M Ω cm. A β (1–42) stock solutions (0.5 mM) were prepared daily as in our previous studies.^{37, 38} Briefly, the lyophilized A β (1–42) was dissolved in 10 mM NaOH solution. Upon sonication for 1 min, the solution was centrifuged at 13000 rpm for 30 min and the supernatants were pipetted out and used for the experiments.

Adsorption Kinetic Study—All the adsorption kinetic measurements were conducted in a LB trough (Nima Technologies Inc., Coventry, UK). Aliquots of A β stock solution were mixed with 200 mL of 5 mM phosphate buffer (pH 7.4) to desired concentrations. Upon mixing, the solution was immediately poured into the LB trough, and the pressure reading was adjusted to zero. The surface pressure variation was monitored over different lengths of time.

Atomic Force Microscopy—AFM images were obtained on a PicoScan SPM microscope (Agilent Technologies, Tempe, AZ) equipped with a magnetic alternating current mode. This mode has proven advantageous for measuring soft biological samples. We have used it to measure aggregation of α -synuclein and SMA proteins^{39, 40} and A β peptides. The AFM substrates were silicon wafers (Mishubishi Silicon America, Salem, OR) thoroughly cleaned by following a literature procedure.⁴¹ For imaging, the samples were prepared by slowly dipping/pulling silicon wafer slides at 2 mm/min into/out of the solution in the LB trough with A β layers formed at different stages of adsorption. The samples were then gently washed with water and dried by nitrogen gas prior to the AFM imaging.

Attenuated Total Reflection-FT-IR Spectroscopy—The Au substrates used for ATR-FTIR characterization of the A β (1–42) films transferred from the air/solution interface are fabricated by evaporating 50-nm thick Au onto clean BK7 glass cover slides using an electron beam evaporation system (CHA Industries, Fremont, CA). After deposition from the LB trough, the samples are dried overnight before IR analysis. The dried protein films are sampled using a single-bounce diamond attenuated total reflectance (ATR) (MIRacle, Pike Technologies, Madison, WI) accessory. Infrared spectra are measured with an unpolarized Mattson Infinity 60AR (formerly Madison, WI) FT-IR at room temperature. A liquid nitrogen cooled mid-band Mercury-Cadmium-Telluride detector (Judson

Technologies, Montgomery, AL) is employed. For each spectrum, 500 scans are signal-averaged for each single beam interferogram with a resolution of 1 cm^{-1} . The interferogram data is Fourier transformed with two zero-fills using Hanning apodization. Reference spectra are recorded using the same ATR accessory in dry air. ATR transmittance spectra are sample single beam spectra ratioed to reference single beam spectra and converted to percent. A digital spectrum of water vapor was subtracted interactively from some samples to compensate for inadequate purging of the IR beam path.

Circular Dichroism (CD) Spectroscopy—CD spectra were recorded on a JASCO J 815 spectrometer using a 0.1 mm path length quartz cell with a 300 LL capacity.

Results

Influence of A β concentration on its adsorption kinetics

Figure 1 shows surface pressure (Π)–time (t) curves for different concentrations of A β in the LB trough. All of the curves exhibit a lag phase, during which the surface pressure remains effectively zero, and a rising phase. Since the rising phase reflects the change in surface pressure and A β is the only “surface-active” species in the trough, the rise of surface pressure is attributed to the adsorption of A β at the air/solution interface. Notice that the surface pressure increases with time until it plateaus. Maltseva and Brezesinski reported analogous surface pressures at the adsorption equilibria ($\sim 12\text{--}16\text{ mN/m}$) of A β , whose bulk concentration ($0.58\text{--}1.16\text{ }\mu\text{M}$ ²⁷) was higher than what we used. Interestingly, the Π – t curves did not display a lag phase, possibly due to the use of a high A β concentration.²⁷ The origin of the lag phase observed at lower A β concentrations will be delineated in the Discussion section. Increasing the A β concentration within the range $0.1\text{--}0.25\text{ }\mu\text{M}$ did not change the overall adsorption behavior as shown in Figure 1. On the other hand, lowering the A β concentration further to $0.05\text{ }\mu\text{M}$ significantly prolongs the lag phase and decreases the adsorption rate. Even at a higher temperature ($30\text{ }^{\circ}\text{C}$) which accelerates the adsorption rate (*vide infra*), the adsorption equilibrium was not observed before a serious loss of solution due to evaporation (Figure 1).

For adsorption that is not diffusion controlled, the following equation can be used for moderate to high surface coverage:^{42, 43}

$$\frac{\Pi_s - \Pi(t)}{\Pi_s - \Pi_0} = \exp(-kt) \quad (1)$$

where $\Pi(t)$ is the surface pressure (mN/m) at a given time t , Π_0 at time 0 or t_0 (the end of the lag phase), Π_s at adsorption equilibrium, and k is the apparent adsorption rate constant. To account for the lag phase, t in eq. 1 can be replaced with $t - t_0$:

$$\Pi(t - t_0) = \Pi_s [1 - \exp(-k(t - t_0))] \quad (2)$$

The good fits between the rising phases of the experimental Π – t curves and the simulated curves (Figure 1) suggest that the kinetics of the A β adsorption is likely not diffusion controlled. Thus, the apparent adsorption rate constant, k , and the duration of the lag phase can be determined for the A β concentrations studied in this work (Table 1).

Notice that k is dependent on the A β bulk concentration, [A β], suggesting that the overall process is not a simple adsorption following its diffusion from the bulk to the surface. Interestingly, the k values were found to increase linearly with [A β] ($R^2 = 0.99$). The process

occurring in the lag phase is apparently slower when $[A\beta]$ is lower, whereas at a higher $[A\beta]$ the process in the rising phase leads to a sharper rise in the surface pressure. These observations suggest that the processes taking place in the lag and rising phases are interdependent. An interpretation of the exact processes and the derivation of the dependence of k on $[A\beta]$ are provided in the Discussion section.

Influence of temperature on the overall $A\beta$ adsorption kinetics

To examine how temperature affects the adsorption kinetics, we recorded a series of Π - t curves at different temperatures. The black and red curves in Figure 2 are again the experimental and partially fitted results, respectively. Similar to the concentration effect, we found that both t_0 and k values vary with temperature. Specifically, a higher temperature shortens the lag phase while accelerates the rising phase. The k and t_0 values at different temperatures are listed in Table 2.

Using the Arrhenius equation, a linear plot of $\ln k$ vs. $1/T$ was obtained (Figure 3), with the activation energy deduced to be 62.2 ± 4.1 KJ/mol. This activation energy is substantially greater than the typical value for diffusion controlled adsorption of surfactants⁴⁴ and globular proteins.⁴⁵ It is about 3 times as great as the energy barrier expected from a diffusion-controlled adsorption process (~ 20 kJ/mol).⁴⁶ Remarkably, the activation energy is rather close to that for lysozyme adsorption (47.2–58.1 kJ/mol).⁴⁶ Moreover, this value is also comparable to that of the adsorption of Lac 28 monomers produced from the dissociation of their tetramers present in the bulk.⁴³ This unusually large activation energy, similar to the concentration dependence of k , suggests that adsorption of $A\beta$ at a low bulk concentration, cannot be ascribed to a simple diffusion-controlled adsorption process. Because Xu and Damodaran suggest that lysozyme could undergo a change in its secondary structure in the subsurface (~ 30 nm underneath the interface)⁴⁶ and the dissociation of the Lac 28 occurred before the adsorption step, it is likely a change also occurs in the case of $A\beta$ prior to its adsorption (*vide infra*).

As natively unstructured $A\beta$ molecules are charged and hydrated, their tendency of adsorbing at the interface is much less than their α -helical counterpart which is less hydrated and more compact. Two experiments were conducted to further confirm this property. In one experiment, we spread $A\beta$ solution freshly prepared in NaOH solution onto the surface of a phosphate buffer solution in the LB trough. Similar to Figure 1, the Π - t curve showed a lag phase (Figure S1 in Supplementary Information). Since NaOH is known to favor the natively unstructured conformation, the fact that the surface pressure did not increase immediately suggests that, instead of remaining at the interface, the $A\beta$ molecules had diffused into the bulk. This is analogous to the initial diffusion of lysozyme from the subsurface to the bulk which is also a hydrophilic and highly charged protein.^{46, 47} In another experiment, $A\beta$ dissolved in trifluoroethanol, a solvent known to favor the α -helical $A\beta$ formation, was spread onto the surface. Interestingly, the surface pressure (Figure S1) rapidly reached the typical equilibrium value. The slight decrease in the surface pressure was caused by either the molecular rearrangement at the interface or a small agitation due to the addition of the solution at the interface. This result suggests that $A\beta$ molecules in the α -helical conformation, unlike the unstructured conformation, remained at the air/solution interface. These two experiments demonstrate that α -helical $A\beta$ is likely the species that adsorbs at the interface.

FT-IR, CD, and AFM characterizations of $A\beta$ monolayers formed at the air/solution interface

To probe the exact secondary structure of the $A\beta$ adsorbates, we resort to ATR-IR and CD spectroscopy. Shown in Figure 4 is an overlay of four ATR-IR spectra of $A\beta$ films

transferred onto thin gold substrates at different stages of the Π - t curve (denoted by numbers next to the curve in the inset). The absorption band at 1662 cm^{-1} (amide I) is close to that of an α -helical structure (1653 cm^{-1}).^{48, 49} Such a small shift may be caused by the removal of water in the dry protein film.⁵⁰ Fitting the amide I band indicates that the α -helical structure predominates in the film which also contains a small amount of the β -sheet structure (see Figure S2). This finding demonstrates that the α -helical structure populates the interface. The presence of the β -sheet structure may suggest that some α -helical A β molecules have converted to the β -sheet structure at the interface or during the film transfer for the subsequent IR analysis. The observation of α -helices supports the *in vitro* aggregation studies that the formation of β -sheet aggregates proceeds through the α -helical A β intermediate.^{51, 52} It is apparent from Figure 4 that the peak intensity corresponding to the A β film produced at a lower surface pressure is less than that of the film transferred at the equilibrium. We also found that incubating an A β solution for an extended period of time (e.g., 3 days) did not increase the amount of A β adsorbates, and bands characteristic of the β -sheet structure were difficult to discern. These observations confirm that the adsorbed A β molecules are present largely in the α -helical conformation and remain relatively stable at the interface.

To rule out that the α -helices observed by the ATR-IR measurement are caused by transfer of the A β molecules from the interface to the Au substrate, we conducted CD measurements of solutions collected from the top layer of the solution in the LB trough. Figure S3 shows a representative CD spectrum which also displays bands (i.e., 193, 210, and 222 nm) close to characteristic bands (i. e. 190, 208, and 222 nm) of α -helices. Such a CD spectrum is in sharp contrast to that corresponding to the natively unstructured A β sample collected from the bulk of the solution.

Interestingly, the secondary structure of A β at the interface formed from a low A β concentration and at physiological pH (7.4) is different from that reported by Brezesinski's group.^{27, 29} In their work the A β film formed from a higher A β concentration possessed a β -sheet structure. Moreover, pure water whose pH could be considerably less than 7 due to dissolved CO₂ was used in their work. It has been reported that a pH closer to the pI of A β (5.5)⁵³ and a higher A β concentration both favor the β -sheet formation. The observation of α -helical A β in our work suggests that the experimental conditions play an important role in altering the formation of a specific A β secondary structure and possibly the mechanism and kinetics of A β adsorption.

Finally, we carried out AFM imaging of the A β films transferred at different stages of the Π - t curve (B, C, and D corresponding to stages 1, 3 and 4; *cf.* inset of Figure 4). We used silicon substrates because they are atomically flat (variation in the cross-sectional contour shown below Figure 5A is $< 0.5\text{ nm}$). When an A β film at a surface pressure of *ca.* 5.5 mN/cm was transferred, the height variation increased to *ca.* 0.75 nm (Figure 5B) and features observed are typically of a few nanometer wide (*cf.* cross-sectional contour). The height increases to $\sim 1\text{ nm}$ for a film transferred at 12.0 mN/cm (Figure 5C) and further to $\sim 3\text{ nm}$ at 12.0 mN/cm (Figure 5D). As the A β monomer is too small to be observed by AFM, these large features should result from the agglomeration of monomers, suggesting that the surface coverage at the air/solution interface is rather high. Such a conclusion supports the validity of using Eq. 1 (for relatively high surface coverage) to fit the Π - t curves in Figures 1 and 2. Recent studies on the synthesis of bionanomaterials from synthetic peptides have shown that α -helical peptides can entangle to form large stable helical assemblies.^{54, 55} As the adsorption proceeds for a longer time, it is also possible that multilayers of α -helical A β are produced at the interface and/or subsurface.

Discussion

The major findings can be summarized as (1) the deviation of the A β adsorption from a diffusion-controlled process, (2) the presence of a lag phase in the Π - t curve, (3) the conformation change of A β near the air/solution interface, and (4) the concentration dependence of the apparent adsorption rate constant and the unusually large adsorption activation energy. Below we discuss these findings in terms of physical processes that could occur throughout the adsorption, *viz.*, the diffusion of A β from the bulk to the subsurface, A β conformation change within the subsurface, and the A β adsorption from the subsurface to the interface.

Diffusion of A β from the bulk to the subsurface

It is generally accepted that the creation of a fresh interface produces a concentration gradient which drives the diffusion of molecules from the bulk and the subsequent adsorption at the interface.^{46, 56} Transfer of a species from the bulk can be realized via diffusion, convection, or a combination of both. In most cases, adsorption of proteins and surfactants at the interface has been found to be diffusion-controlled.^{57, 58} Any deviation from diffusion-controlled behavior has been ascribed to the presence of an energy barrier caused by the sluggish adsorption step^{43, 47, 59–61} or a large apparent activation energy barrier associated with chemical reactions/structural variation of the adsorbing species en route to their final adsorption at the interface.^{46, 62}

For a diffusion-controlled adsorption process, the characteristic diffusion time, t_d , of A β from the bulk to the interface can be estimated using the following equation, which is extrapolated from a linear isotherm under the semi-infinite diffusion condition:⁶³

$$t_d = \frac{3.1416}{4D} \left(\frac{\Gamma_{\max}}{[A\beta]} \right)^2 \quad (3)$$

where D is the A β diffusion coefficient in the bulk ($1.25 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$)⁶⁴ and $\Gamma_{\max}$ is the maximum A β surface concentration (coverage). Because $\Gamma_{\max}$ is approached asymptotically and t_d would be infinite at saturation coverage,⁶⁵ we used the time corresponding to 95% of the highest Γ recorded in the curves in Figure 1 as the measured t_d value. It is well documented that α -helical peptides orientate in parallel to interface once adsorbed.^{27, 43, 66, 67} Since our ATR-IR and CD measurements have both indicated that A β molecules at the interface are in the α -helical conformation, we presume that the A β molecules are also aligned with their helical axes parallel to the interface. Each helical residue gives a projected equivalent cross section area of $35 \text{ \AA}^2$.²⁴³ In a relatively hydrophobic environment (e.g., that within the subsurface), A β is expected to fold into three helical segments, located between residues 15–24, 28–36, and 37–42. These segments yield a total cross section area of $770 \text{ \AA}^2$ or $\Gamma_{\max} = 2.15 \times 10^{-11} \text{ mol cm}^{-2}$. Therefore, the typical t_d value at $[A\beta] = 0.25 \text{ }\mu\text{M}$ is about 4,600 s, which is appreciably shorter than that measured experimentally ($\sim 9,500 \text{ s}$ or $11,500 \text{ s}$ if the lag phase is included in Figure 1). If natural convection also contributes to the mass transfer, the discrepancy will even be greater. Interestingly, for curves exhibiting a lag phase, t_d is still shorter than or at the most comparable to the adsorption time that also includes the duration of the lag phase. For example, t_d for $[A\beta] = 0.1 \text{ }\mu\text{M}$ was deduced to be 29,000 s, whereas the experimental time for reaching equilibrium is approximately the same. Therefore, we conclude that under the experimental conditions, the overall adsorption process is *not* diffusion controlled. Since the diffusion can keep up with the A β adsorption rate, it becomes understandable that the equilibrium coverage approaches the maximum surface coverage, a point drawn from the

successful fit of the experimental data using eq. 1 (*vide supra*) and our aforementioned AFM results.

Conformation change of A β within the subsurface

It is widely recognized that there exists a layer below the surface (i.e., subsurface) whose thickness is dependent on the solution composition (e.g., ionic strength) and the concentration and properties of the adsorbate. For protein adsorbates, charges residing at the exterior and the cumulative hydrophobicity/hydrophilicity contributed by the amino acid residues can affect the subsurface thickness. Xu and Damodaran⁴⁶ have suggested that a more accurate term to describe the protein adsorption at the interface is the chemical potential gradient ($\Delta\mu$). In their studies of adsorption of lysozyme from a phosphate buffered saline solution to the air/solution interface, they attributed the origin of a downward parabolic lag phase to the $\Delta\mu$ in the subsurface. Although these researchers did not provide spectroscopic evidence for the lysozyme conformation change, they demonstrated that pre-denaturation (pre-unfolding) of the lysozyme molecules resulted in the disappearance of the lag phase and the acceleration of the overall adsorption kinetics. Middelberg *et al.*⁴³ reported an interesting system in that the Lac 28 tetramer dissociation prior to the adsorption step contributes significantly to the apparent adsorption energy barrier. Although the conformation change suggested by Xu and Damodaran⁴⁶ is a physical process whereas the tetramer dissociation in Middelberg's work is a chemical reaction, both precede the adsorption process and affect the overall kinetics. These characteristics have some similarity to our kinetic model described below.

In the present study, we have obtained concrete spectroscopic evidence for the change in the A β secondary structure happening within the subsurface. Moreover, analogous to the studies performed by Xu and Damodaran,^{46, 47} we have shown that the preformed α -helical A β molecules stay at the interface (*cf.* Figure S1). The presence of such a lag phase cannot be attributed to the low A β bulk concentration, since we have mentioned earlier that diffusion is a relatively fast process. This is further supported by the finding that globular proteins present at even lower concentrations (~nanomolar level^{68, 69}) do not produce a lag phase. Therefore, we ascribe the lag phase to the development of a dynamic equilibrium between the A β conformational states, which concomitantly modulates the hydrophobic and electrostatic interactions within the subsurface. Such equilibrium is also intricately related to the subsequent adsorption step. All of these processes affect the overall chemical potential gradient within the subsurface:

$$\mu = \mu_{conc} + \mu_{conf} + \mu_{hydro} + \mu_{ele} \quad (4)$$

where μ_{conc} is the chemical potential contributed by the concentration gradient, μ_{conf} is the contribution from conformation change due to entropy change and other processes such as hydration/dehydration and hydrogen bond formation/breakage, μ_{hydro} is associated with the hydrophobic force on the protein surface with its surrounding, and μ_{ele} is resulted from the electrostatic interaction between charged peptides. Since in our case, the overall adsorption of A β at the interface is not controlled by diffusion, we here consider mainly the contributions to the cumulative μ by forces that may vary during the conformation change.

μ_{ele} contributed by charges on A β molecules in the subsurface can be estimated using the following equation:⁴⁶

$$\mu_{ele} = ee' / 2d\epsilon = (e^2 / 2d\epsilon) \{(\epsilon - \epsilon_0) / (\epsilon_0 + \epsilon)\} \quad (5)$$

where e is the overall charge on the surface of A β , e' is the image charge, and ϵ and ϵ_0 are the dielectric constants of the aqueous and gas phases, respectively, and d is the thickness of the subsurface. At physiological pH, the net charge on a natively unstructured A β molecule is about -3 .⁷⁰ Such a charge would give a thickness of ~ 2.5 nm when equating eq.3 to the thermal energy kT (k is the Boltzmann constant) and assuming that the electrostatic interaction is the major contributor to μ . It is interesting to note that this thickness is comparable to the dielectric layer thickness suggested to be near a cell membrane.^{30, 31}

At the beginning of the experiment A β molecules in the subsurface change their conformation to adapt to the hydrophobic environment, which will create a concentration gradient of unstructured A β (from the bulk to the subsurface). At the same time, the concentration of the α -helical A β is higher than that in the bulk (i.e., direction of the gradient opposite of that of unstructured A β). As suggested by Xu and Damodaran,⁴⁶ at some distance away from the interface, the forces shown in eq. 4 will be balanced and the overall chemical potential gradient will be zero across this distance. Since most of the charged groups reside near the A β N-terminal which remains unfolded in the α -helical conformation,^{22, 34} it is likely that folding of the natively unstructured A β does not cause a significant change in μ_{ele} . Therefore, the major variations in chemical potential ($\Delta\mu$) upon A β conformation change arise from the accumulation of the A β molecules in the α -helical conformation (μ_{conc}), and the A β conformation change (μ_{conf}) which exposes more hydrophobic residues to the interface to lower the interfacial free energy. Therefore, in the subsurface, $\Delta\mu_{\text{conc}}$ is positive for the α -helical A β , whereas $\Delta\mu_{\text{hydro}}$ and $\Delta\mu_{\text{conf}}$ are negative due to the A β folding which stabilizes A β in the subsurface. Notice from the above discussion that changes in some of the terms in eq. 4 caused by the conformation change can be different than those associated with the diffusion process. Overall, across the subsurface, the cumulative chemical potential gradient remains unchanged for a period of time after the A β changes its conformation, resulting in the accumulation of α -helical A β in the subsurface and continued diffusion of A β continues from the bulk. This leads to a continuous conversion of the unstructured to the α -helical conformation without causing any observable interfacial pressure change. Similar to the lysozyme adsorption process,⁴⁶ the temporary balance between the positive and negative driving forces may be the origin of the lag phase. It then becomes understandable why the A β bulk concentration affects the duration of the lag phase. Since diffusion is relatively facile, a higher A β bulk concentration should lead to a more rapid buildup of the α -helical concentration across the subsurface, disrupting the balance earlier among the various forces.

Adsorption of α -helical A β molecules from the subsurface to the interface

The presence of a lag phase also suggests that the energy barrier for the adsorption of α -helical A β to the interface must be relatively high. As abovementioned, the adsorption activation energy for proteins is typically ~ 20 kJ/mol, substantially less than our experimentally measured value (62 kJ/mol). The free energy of unstructured A β in the bulk should be much greater than that of the adsorbed A β . However, the difference in free energy between the α -helical A β in the subsurface and that at the interface is much less. Thus, it appears that the accumulation of α -helical A β within the subsurface is a prerequisite for the final adsorption. As described in connection to the origin of the lag phase, the continuous buildup of α -helical A β molecules within the subsurface will eventually make the positive contributions to the chemical potential outweigh the negative contributions.

Kinetic model and adsorption rate equation

Taken together, the overall A β adsorption can be described by the schematic diagram shown in Fig. 6. As unstructured A β moves to the subsurface, the following are at work: the hydrophobic force facilitates the A β conformation change, however such a force also

stabilizes the resultant α -helical A β . At the same time, the buildup of the α -helical A β molecules increases the concentration gradient between the subsurface and interface and that between the subsurface and the bulk. The diffusion of α -helical A β from the subsurface to the bulk is thermodynamically disfavored but that to the interface is favored. However, the existence of an adsorption activation energy barrier resulted from the rearrangement of α -helical A β molecules and further dehydration of the molecules retards the adsorption of α -helical A β to the interface. Before the adsorption of the accumulated α -helical A β molecules, the opposite forces are in a dynamic equilibrium and no appreciable change in the surface pressure is observed. Ultimately, the balance in the chemical potential gradient is disrupted and the propensity of α -helical A β to the interface results in adsorption. Overall, the lag phase should be related (or correspond) to the establishment of the conformational transformation equilibrium of A β . During the adsorption phase, the supply of unstructured A β from the solution to the subsurface and the removal of α -helical via surface adsorption continue to occur.

The diffusion of unstructured A β from the bulk ($A\beta_{sol}^{un}$) to the subsurface, the equilibrium between the unstructured ($A\beta_{sub}^{un}$) and the α -helical A β ($A\beta_{sub}^{\alpha}$) molecules within the subsurface, and the adsorption which yields $A\beta_s^{\alpha}$ at the interface can be summarized as follows:

where k_f and k_r are the forward and reverse reaction rate constants and k_{ad} is the adsorption rate constant. Notice that the desorption step is neglected because protein adsorption at a surface is almost always irreversible.^{43, 59, 62} Moreover, some hydrophobic residues of the adsorbed A β may have protruded into the air (Figure 6a), making the reverse process difficult.

Since the overall kinetics is governed by the adsorption step, the following rate equation holds:

$$\frac{d}{dt} \left(\frac{\Pi}{\Pi_{\max}} \right) = k_{ad} [A\beta_{sub}^{\alpha}] (1 - \theta) = k_{ad} K [A\beta_{sub}^{un}] (1 - \theta) \approx k_{ad} K [A\beta_{sol}^{un}] (1 - \theta) \quad (7)$$

where K is the equilibrium constant which is equal to k_f/k_r and θ is the fractional surface coverage which equals $\Pi/\Pi_{\max}$ or $\Gamma/\Gamma_{\max}$.

Rewriting eq.7 in terms of the relative surface pressure change, the following equation can be derived:

$$\frac{d}{dt} \left(\frac{\Pi}{\Pi_{\max}} \right) = \frac{d\theta}{dt} = k_{ad} K [A\beta_{sol}^{un}] (1 - \theta) \quad (8)$$

Solving for θ in eq. 8 gives:

$$\theta(t) = \frac{\Pi}{\Pi_{\max}} = 1 - \exp(-k_{ad} K [A\beta_{sol}^{un}] t) \quad (9)$$

If the duration of the lag phase (t_0) is accounted for, eq. 9 can be expressed as:

$$\theta(t) \frac{\Pi}{\Pi_{\max}} = 1 - \exp(-k_{ad}K [A\beta_{sol}^{un}](t - t_0)) \quad (10)$$

It is therefore obvious that eq. 10 and eq. 2 are equivalent, and the apparent rate constant k in eq. 2 is equal to $k_{ad}K [A\beta_{sol}^{un}]$. This relationship quantitatively explains why the apparent adsorption rate constant k is proportional to the $A\beta$ bulk concentration.

Similar to the case of Lac 28 tetramer adsorption to the air/water interface, the unusually high adsorption activation energy measured here may be related to a preadsorption equilibrium in the subsurface. The good agreement between the experimental data and the kinetic model derived above based on the presence of a conformational transformation equilibrium substantiates the accumulation of α -helical $A\beta$ molecules within the subsurface. Based on the rate equation deduced, the apparent adsorption rate constant equals $k_{ad} \times K$. Thus, the apparent activation energy includes the free energy change of the preadsorption equilibrium process and the activation energy of the adsorption process. This may explain the unusually high apparent activation energy value measured here.

Biological implications

Our findings have several important implications to the $A\beta$ aggregation process. First of all, the fact that $A\beta$ can be adsorbed onto an interface and undergoes a conformation change at a submicromolar level suggests that it is possible for $A\beta$ to aggregate *in vitro* at a concentration much closer to the *in vivo* $A\beta$ concentration. That $A\beta$ changes its conformation near an interface without adsorbing at the surface may be relevant to the development of AD, as it is known that in brain diffuse plaques of $A\beta$ exist long before the appearance of senile plaques.⁷¹ Nevertheless, a higher $A\beta$ concentration will accelerate the conformation change and enhance the adsorption step. This is in line with observations that overproduction of $A\beta$ due to gene mutation and metabolic dysfunctions to rid $A\beta$ of the cellular milieu are two of the major causes for amyloidosis. Second, it appears from our study that at least one of the roles played by an interface is to facilitate the $A\beta$ conformation change before its adsorption. The role of a heterogeneous surface in the commonly used nucleation-polymerization theory is to provide seeds for initiating the aggregation process.⁷² As mentioned in the Introduction, there exists a layer near a cell membrane whose hydrophobicity is much greater than that of the extracellular matrix. Our study suggests that such a hydrophobic layer could help accumulate $A\beta$ and even facilitate the $A\beta$ misfolding and the follow-up aggregation/fibrillation. We should also mention that for *in vitro* production of aggregates of amyloidogenic proteins (e.g., α -synuclein), the presence of a hydrophobic seed (e.g., a Teflon bead) drastically promotes the aggregation rate.⁷³ Third, from our study, it is clear that an induction time (lag phase) is also affected by other factors such as the $A\beta$ monomer structure (which in turn modulates the chemical potential near the interface), the solution composition, and temperature. Any neuropathological effects could alter these factors and subsequently shorten the induction time. For example, a pH decrease will annihilate the $A\beta$ surface charge (i.e., a reduced μ_{ele}), which in turn will increase its propensity to accumulate at and adsorb onto the cell membrane (i.e., an increased μ_{hydro}). Likewise, metal binding^{74, 75} changes the $A\beta$ surface charge and structure, both of which may also alter its adsorption behavior. In common with the appearance of $A\beta$ deposition is the observation that altered neuronal H^+ homeostasis may accompany AD acidosis and brain injury.^{76, 77} Fourth, increasing temperature will have a large effect on the adsorption process, accelerating the deposition of either $A\beta$ monomers or species of higher masses. Recently, high body temperature caused by inflammation has been connected to AD.⁷⁷ All

of these observations may be linked to the development of AD. Although our results cannot differentiate the sequence throughout the aggregation process (e.g., aggregation followed by adsorption vs. adsorption prior to aggregation), the unusually large adsorption energy barrier suggests that, without a conformation change and an accumulation at the interface, deposition of A β monomers is kinetically disfavored. Finally, the kinetic model based on an equilibrium followed by the adsorption step is generic and should be extendable to the studies of other protein adsorption processes wherein a lag phase is observed.

Conclusion

We have demonstrated that kinetic study of the A β adsorption at the air/solution interface provides valuable insight into the A β conformation change and accumulation at surfaces. Our results indicate that the movement of A β from a very dilute solution is driven by the hydrophobic layer (subsurface) adjacent to a heterogeneous interface which favors the formation of folded A β conformation(s). The adsorption of A β whose structure is different than its unstructured variant must overcome a high energy barrier (62.2 ± 4.1 KJ/mol). As a result, A β accumulates within this layer and establishes an equilibrium between two different conformational states. The presence of the lag phase can be qualitatively interpreted using the variations of chemical potentials associated with electrostatics, conformation change, hydrophobic force, and concentration gradients of A β . At the quantitative level, the kinetic model based on this mechanism allowed us to derive the adsorption rate equation, which shows the dependence of the apparent adsorption rate constant on the A β bulk concentration. The high adsorption energy barrier also suggests that the conformation change within the hydrophobic subsurface stabilizes an A β structure whose exterior is more hydrophobic. As a consequence, a substantial local chemical potential gradient needs to be developed before A β adsorbs at the interface. Therefore, the effect of temperature is expected to have a more pronounced effect on the adsorption step. Judging from the terms affecting the chemical potential gradient between the bulk solution and the subsurface and that between the subsurface and the interface (*cf.* eq. 4), it can be envisaged that various parameters, such as pH, ionic strength, temperature of the solution, other species that also affect A β structure and properties (e.g., metal ions), and concentration of A β , can all have profound influences on the forces contributing to the interfacial chemical potential. Therefore, they will dictate the course of A β misfolding, accumulation, and aggregation. Indeed, many of these factors have been confirmed to be important in the A β aggregation/fibrillation pathways by other *in vitro* and *in vivo* studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Reference

1. Hardy J, Selkoe DJ. Science. 2002; 297:353–356. [PubMed: 12130773]
2. Selkoe DJ. Science. 1997; 275:630–631. [PubMed: 9019820]
3. Kang J, Lemaire HG, Unterbeck A, Salbaum JM, Masters CL, Grzeschik KH, Multihaupt G, Beyreuther K, Muller-Hill B. Nature. 1987; 325:733–736. [PubMed: 2881207]
4. Wang R, Sweeney D, Gandy S, Sisodia S. J. Biol. Chem. 1996; 271:31894–31902. [PubMed: 8943233]

5. Lewczuk P, Esselmann H, Groemer TW, Bibl M, Maler JM, Steinacker P, Otto M, Kornhuber J, Wiltfang J. *Biol. Psych.* 2003; 55:524–530.
6. Southwick PC, Yamagata SK, Echols CL Jr, Higson GJ, Neynaber SA, Parson RE, Munroe WA. *J. Neurochem.* 1996; 66:259–265. [PubMed: 8522962]
7. Haass CB, Schlossmacher MG, Hung AY, Vigo-Pelfrey C, Mellon A, Ostaszewski BL, Lieberburg I, Koo EH, Schenk D, Teplow DB. *Nature.* 1992; 359:322–325. [PubMed: 1383826]
8. Shoji M, Glode TE, Ghiso J, Cheung TT, Estus S, Shaffer LM, Cai XD, McKay DM, Tintner R, Frangione B, Younkin SG. *Science.* 1992; 258:126–129. [PubMed: 1439760]
9. Suebert P, Vigo-Pelfrey C, Esch F, Lee M, Dovey H, Davis D, Sinha S, Schlossmacher MG, Whaley J, Swindlehurst C, McCormack R, Wolfert R, Selkoe DJ, Lieberburg I, Schenk D. *Nature.* 1992; 359:325–327. [PubMed: 1406936]
10. Hardy JA, Higgins GA. *Science.* 1992; 256:184–185. [PubMed: 1566067]
11. Sengupta P, Garai K, Sahoo B, Shi Y, Callaway DJ, Maiti S. *Biochemistry.* 2003; 42:10506–10513. [PubMed: 12950178]
12. Gorman PM, Yip CM, Fraser PE, Chakrabartty A. *J. Mol. Biol.* 2003; 325:743–757. [PubMed: 12507477]
13. Lomakin A, Chung DS, Benedek GB, Kirschner DA, Teplow DB. *Proc. Natl. Acad. Sci. USA.* 1996; 93:1125–1129. [PubMed: 8577726]
14. Lomakin A, Teplow DB, Kirschner DA, Benedek GB. *Proc. Natl. Acad. Sci. USA.* 1997; 94:7942–7947. [PubMed: 9223292]
15. Yong W, Lomakin A, Kirkitadze MD, Teplow DB, Chen S-H, Benedek GB. *Proc. Natl. Acad. Sci. USA.* 2002; 99:150–154. [PubMed: 11756677]
16. Bokvist M, Gröner G. *J. Am. Chem. Soc.* 2007; 129:14848–14849. [PubMed: 17990885]
17. Terzi E, Holzemann G, Seelig J. *J. Mol. Biol.* 1995; 252:633–642. [PubMed: 7563079]
18. Terzi E, Holzemann G, Seelig J. *Biochemistry.* 1997; 36:14845–14852. [PubMed: 9398206]
19. Yip CM, Darabie AA, McLaurin J. *J. Mol. Biol.* 2002; 318:97–107. [PubMed: 12054771]
20. Yamaguchi H, Maat-Schieman MLC, Duinen SG, Prins FA, Neeskens P, Natta R, Roos RAC. *J. Neuropathol. Exp. Neurol.* 2000; 59:723–732. [PubMed: 10952062]
21. Kowalewski T, Holtzman DM. *PNAS.* 1999; 96:3688–3693. [PubMed: 10097098]
22. Coles M, Bicknell W, Watson AA, Fairlie DP, Craik DJ. *Biochemistry.* 1998; 37:11064–11077. [PubMed: 9693002]
23. Selkoe D. *Trends Neurosci.* 1993; 16:403–409. [PubMed: 7504355]
24. Hilbich C, Kisters-Woike B, Reed J, Masters CL, Beyreuther K. *J. Mol. Biol.* 1991; 218:149–163. [PubMed: 2002499]
25. Barrow CJ, Zagorski MG. *Science.* 1991; 253:179–182. [PubMed: 1853202]
26. Schladitz C, Vieira EP, Hermal H, Mohwald H. *Biophys. J.* 1999; 77:3305–3310. [PubMed: 10585952]
27. Maltseva E, Brezesinski G. *ChemPhysChem.* 2004; 5:1185–1190. [PubMed: 15446741]
28. Soreghan B, Kosmoski J, Glabe C. *J. Biol. Chem.* 1994; 269:28551–28554. [PubMed: 7961799]
29. Maltseva E, Kerth A, Blume A, Mohwald H, Brezesinski G. *ChemBioChem.* 2005; 6:1817–1824. [PubMed: 16175542]
30. Teschke O, de Souza EF. *Chem. Phys. Lett.* 2005; 403:95–101.
31. Cherepanov DA, Feniouk BA, Junge W, Mulikjanian AY. *Biophys. J.* 2003; 85:1307–1316. [PubMed: 12885673]
32. Shen C-L, Murphy RM. *Biophys. J.* 1995; 69:640–651. [PubMed: 8527678]
33. Kim JR, Gibson TJ, Murphy RM. *Biotechnol. Prog.* 2006; 22:605–608. [PubMed: 16599584]
34. Marciniowski KJ, Shao H, Clancy EL, Zagorski MG. *J. Am. Chem. Soc.* 1998; 120:11082–11091.
35. Li C, Orbulescu J, Sui G, Leblanc RM. *Langmuir.* 2004; 20:8641–8645. [PubMed: 15379486]
36. Triulzi RC, Li C, Naistat D, Orbulescu J, Leblanc RM. *J. Phys. Chem. C.* 2007; 111:4661–4666.
37. Jiang D, Men L, Wang J, Zhang Y, Chickenyen S, Wang Y, Zhou F. *Biochemistry.* 2007; 46:9270–9282. [PubMed: 17636872]

38. Maitti NC, Jiang D, Wain AJ, Patel S, Dinh KL, Zhou F. *J. Phys. Chem. B.* 2008; 112:8406–8411. [PubMed: 18570397]
39. Zhu M, Han S, Zhou F, Millet IS, Fink AL. *J. Biol. Chem.* 2004; 279:24452–24459. [PubMed: 15056656]
40. Zhu M, Rajamani S, Kaylor J, Han S, Zhou F, Fink AL. *J. Biol. Chem.* 2004; 279:26846–26857. [PubMed: 15096521]
41. Sehell NB, Petrash S, Foster MD, Tsukruk VV. *Langmuir.* 1998; 14:4535–4544.
42. Graham DE, Phillips MC. *J. Colloid Interface Sci.* 1979; 70:403–414.
43. Middelberg APJ, Radke CJ, Blanch HW. *PANS.* 2000; 97:5054–5059.
44. Pogorzelski SJ, Kogut AD. *Oceanologia.* 2001; 43:389–404.
45. Wierenga PA, Meinders MJB, Egmond MR, Voragen AGJ, de Jongh HHJ. *J. Phys. Chem. B.* 2005; 109:16946–16952. [PubMed: 16853156]
46. Xu S, Damodaran S. *Langmuir.* 1992; 8:2021–2027.
47. Xu S, Damodaran S. *Langmuir.* 1994; 10:472–480.
48. Krimm S, Bandekar J. *Adv. Protein Chem.* 1986; 38:181–364. [PubMed: 3541539]
49. Dousseau F, Pezolet M. *Biochemistry.* 1990; 29:8771–8779. [PubMed: 2271555]
50. Bramanti E, Benedetti E, Nicolini C, Berzina T, Erokhin V, D'Alessio A, Benedetti E. *Biopolymers.* 1998; 42:227–237. [PubMed: 9235001]
51. Walsh DM, Hartley DM, Kusumoto Y, Fezoui Y, Condrón MM, Lomakin A, Benedek GB, Selkoe DJ, Teplow DB. *J. Biol. Chem.* 1999; 274:25945–25952. [PubMed: 10464339]
52. Kirkitadze MD, Condrón MM, Teplow DB. *J. Mol. Biol.* 2001; 312:1103–1119. [PubMed: 11580253]
53. Fezoui Y, Hartley DM, Harper JD, Khurana R, Walsh DM, Condrón MM, Selkoe DJ, Lansbury PT Jr, Fink AL, Teplow DB. *Amyloid: Int. J. Exp. Clin. Invest.* 2000; 7:166–178.
54. Potekhin SA, Melnik TN, Popov V, Lanina NF, Vazina AA, Rigler P, Verdini AS, Corradin G, Kajava AV. *Chem. Biol.* 2001; 8:1025–1032. [PubMed: 11731294]
55. Kajava AV, Potekhin SA, Corradin G, Leapman R. *J. Peptide Sci.* 2004; 10:291–297. [PubMed: 15160841]
56. Ward AFH, Tordai L. *J. Chem. Phys.* 1946; 14:453–461.
57. MacRitchie F, Alexander AE. *J. Colloid Interface Sci.* 1963; 18:453–456.
58. Benjamins J, De Feijter JA, Evans MTA, Graham DE, Phillips MC. *Faraday Discuss. Chem. Soc.* 1975; N59:218–229. [PubMed: 1231999]
59. MacRitchie F, Alexander AE. *J. Colloid Interface Sci.* 1963; 18:464–469.
60. Damodaran S, Song KB. *Biochim. Biophys. Acta.* 1988; 954:253. [PubMed: 3370217]
61. Lin S-Y, McKeigue K, Maldarelli C. *Langmuir.* 1991; 7:1055–1066.
62. Narsimhan G, Uraizee F. *Biotechnol. Prog.* 1992; 8:187–196. [PubMed: 1368257]
63. Makievski AV, Fainerman VB, Miller R, Bree M, Liggieri L, Ravera F. *Colloids. Surf.* 1997; 122:269–273.
64. Danielsson J, Jarvet J, Damberg P, Graslund A. *Biochemistry.* 2004; 43:6261–6269. [PubMed: 15147210]
65. Delahay P, Trachtenberg I. *J. Am. Chem. Soc.* 1956; 79:2355–2362.
66. Boncheva M, Vogel H. *Biophys. J.* 1997; 73:1056–1072. [PubMed: 9251822]
67. Caessens PWJR, De Jongh HHJ, Norde W, Gruppen H. *Biochim. Biophys. Acta.* 1999; 1430:73–83. [PubMed: 10082935]
68. Makievski AV, Fainerman VB, Bree M, Wiistneck R, Kragel J, Miller R. *J. Phys. Chem. B.* 1998; 102:417–425.
69. Fainerman VB, Lylyk SV, Ferri JK, Miller R, Watzkle H, Leser ME, Michel M. *Colloids. Surf. A: Physicochem. Eng. Aspects.* 2006; 282:217–221.
70. Flock D, Colacino S, Colombo G, Nola AD. *Proteins: Structure, Function, and Bioinformatics.* 2006; 62:183–192.

71. Huang THJ, Yang D-S, Fraser PE, Chakrabartty A. *J. Biol. Chem.* 2000; 275:36436–36440. [PubMed: 10961999]
72. Yip CM, McLaurin J. *Biophys. J.* 2001; 80:1359–1371. [PubMed: 11222297]
73. Zhu M, Souillac PO, Ionescu-Zanetti C, Carter SA, Fink AL. *J. Biol. Chem.* 2002; 277:50914–50922. [PubMed: 12356747]
74. Karr JW, Kaupp LJ, Szalai VA. *J. Am. Chem. Soc.* 2004; 126:13534–13538. [PubMed: 15479110]
75. Syme CD, Nadal RC, Rigby SEJ, Viles JH. *J. Biol. Chem.* 2004; 279:18169–18177. [PubMed: 14978032]
76. Yates CM, Butterworth J, Tennant MC, Gordon A. *J. Neurochem.* 1990; 55:1624–1630. [PubMed: 2213015]
77. Atwood CS, Moir RD, Huang X, Scarpa RC, Bacarra NME, Romano DM, Hartshorn MA, Tanzi RE, Bush AI. *J. Biol. Chem.* 1998; 273:12817–12826. [PubMed: 9582309]

Figure 1.

Π - t curves at different A β concentrations in the Langmuir-Blodgett trough (in black) and the partially fitted curves (in red). All experiments were performed at 25 $^{\circ}$ C. The A β concentrations are 0.25, 0.20, 0.15, 0.10 and 0.05 μ M. The temperature of the trough was maintained at 25 $^{\circ}$ C for all of the solutions except that the temperature for the 0.05 μ M solution was higher (30 $^{\circ}$ C).

Figure 2.
 Π - t curves recorded at different temperatures (black) and the partially fitted curves for the rising phases (red). The A β concentration in the LB trough was 0.1 μM .

Figure 3.
Plot of $\ln k$ vs. $1/T$ for the adsorption of $A\beta$ onto the air/solution interface.

Figure 4. Overlaid ATR-FT-IR spectra of A β films transferred onto Au substrates at different stages of the Π - t curve. Inset: stages at which the films were transferred are numbered along a typical Π - t curve.

Figure 5. AFM images of a bare silicon wafer (A) and A β films transferred from the air/solution interface at different stages of the Π - t curve (images B–D, corresponding to stages 1, 3, and 4 in the inset of Figure 4, respectively). The bulk A β concentration was 0.1 μ M and the temperature was maintained at 25 $^{\circ}$ C. The cross-sectional contours below the images correspond to representative sampling areas.

Figure 6. Schematic representation of the adsorption process. The α -helical A β is shown to have an unfolded hydrophilic tail.

Table 1Adsorption rate constants and durations of lag phases at different A β concentrations

[A β] (μ M)	k ($s^{-1} \times 10^{-4}$)	t_0 ($s \times 10^3$)
0.10	1.5 ± 0.2	6.0 ± 0.8
0.15	2.7 ± 0.4	5.0 ± 0.7
0.20	4.6 ± 0.3	3.2 ± 0.7
0.25	5.3 ± 0.6	1.8 ± 0.2

Table 2

Adsorption rate constants and durations of lag phases at different temperatures

T (°C)	k ($s^{-1} \times 10^{-4}$)	t_0 ($s \times 10^3$)
37	10.5 ± 0.8	1.2 ± 0.1
30	5.7 ± 0.4	3.4 ± 0.1
25	3.8 ± 0.4	6.0 ± 0.6
20	2.2 ± 0.1	8.1 ± 0.5
14	1.1 ± 0.1	11.0 ± 0.3