Behavior of β-Amyloid 1–16 at the Air–Water Interface at Varying pH by Nonlinear Spectroscopy and Molecular Dynamics Simulations

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ABSTRACT: The adsorption and aggregation of β-amyloid (1–16) fragment at the air–water interface was investigated by the combination of second harmonic generation (SHG) spectroscopy, Brewster angle microscopy (BAM), and molecular dynamics simulations (MD). The Gibbs free energy of surface adsorption was measured to be −10.3 kcal/mol for bulk pHs of 7.4 and 3, but no adsorption was observed for pH 10–11. The 1–16 fragment is believed not to be involved in fibril formation of the β-amyloid protein, but it exhibits interesting behavior at the air–water interface, as manifested in two time scales for the observed SHG response. The shorter time scale (minutes) reflects the surface adsorption, and the longer time scale (hours) reflects rearrangement and aggregation of the peptide at the air–water interface. Both of these processes are also evidenced by BAM measurements. MD simulations confirm the pH dependence of surface behavior of the β-amyloid, with largest surface affinity found at pH = 7. It also follows from the simulations that phenylalanine is the most surface exposed residue, followed by tyrosine and histidine in their neutral form.

INTRODUCTION

Alzheimer’s disease (AD) is a neurological disorder characterized by the formation of insoluble plaques comprising fibrils of aggregated β-amyloid protein.1 The fibrils are thought to be primarily composed of the first 43 residues of the β-amyloid protein as a peptide fragment that has structurally rearranged from a random coil and α-helix conformation into a β sheet.2 The latter half of the β-amyloid peptide (residues 25–35) is sufficient for fibril formation,3 but it is presently unknown how the first half of the β-amyloid fragment is involved in the process. More recent studies, briefly reviewed in ref 6, have implicated the formation of soluble oligomers of β-amyloid, rather than insoluble fibrils, as a key element in the pathogenesis of AD.

Interfaces constitute sites that can catalyze or otherwise influence chemical processes like aggregation. Numerous studies, including recent work investigating ions at the air–water interface, have demonstrated the utility of second harmonic generation (SHG) for probing the surface behavior of ions and molecules.4 SHG is a nonlinear optical process that is the result of two photons of the same energy being converted by a noncentrosymmetric medium into a single photon of twice the energy. This requirement of broken inversion symmetry within the dipole approximation makes SHG a surface-specific technique for bulk centrosymmetric samples, e.g., aqueous solutions, and it can be used to determine interfacial concentrations of molecules through resonant enhancement of the SHG response via their respective absorption spectra. Some earlier SHG studies have been done on proteins at the air–water interface. Girault and others investigated glucose oxidase,5 cytochrome c,6,11 and hemoglobin and myoglobin11 by exploiting the SHG response of specific chromophores, while other groups have examined tryptophan peptides.13–15 SHG circular dichroism has been used to investigate the molecular orientation of proteins at interfaces.16–18 β-Amyloid fibrils form on neurons. Since the water structures at water–hydrophobe and water–air interfaces are thought to be similar, the study of aggregation of β-amyloid at the air–water interface can lend some insight into its interfacial behavior and has previously been investigated experimentally.19–23 Essenthal and Salafsky measured the absorption of β-amyloid 1–40 to a silica–water interface,11 but the quantitative affinity of β-amyloid for an air–water interface, and the role of the first half of the β-amyloid peptide at the interface have not yet been established.4 Also, despite a large body of simulation studies of β-amyloids including their interaction with antiaggregation agents and various interfaces,24–29 the behavior at the water surface has been explicitly addressed only for model amyloids or amyloid-like hydrophobins.30–32 In the present paper we investigate the surface behavior of the first half of the β-amyloid peptide by means of SHG.
For the BAM studies, 1 mg of \( \beta \)-amyloid 1–16 (Anaspec, Inc.) was dissolved in buffer at 1 mg per 25 mL of buffer. To achieve pH 7, two different buffers were used: phosphate buffered saline (0.01 M PBS, which is 0.138 M NaCl, 0.0027 M KCl, 0.008 M \( \text{Na}_2\text{HPO}_4 \), 0.00146 M \( \text{KH}_2\text{PO}_4 \) ; pH 7.4) and citrate (0.05 M citrate, 0.001 M \( \text{NaHPO}_4 \) pH 7). Carbonate buffer (0.05 M \( \text{K}_2\text{CO}_3 \), 0.04 M \( \text{KHCO}_3 \) produced a pH of 10, and pH 3 was achieved with a phosphate buffer (0.05 M \( \text{KHPO}_4 \), 0.006 M HCl). All salts were purchased from Sigma-Aldrich, and the water was produced in a Millipore system generating 18.2 MΩ water. All ions,37 needed to compensate the net charge of the \( \beta \)-amyloid being lost within the experiment, acidic (pH = 3), neutral (pH = 7), and basic (pH = 10) conditions were simulated. In the \( \beta \)-amyloid (i.e., polypeptide \( \text{H}_2\text{N-DAEFRHDSGYEVHHQK-COOH} \)) the titratable groups (i.e., K, R, H, D, E, Y, and the terminal groups) were either charged or neutral according to apparent pK\(_a\) values.33 The protonation states were established for each of the investigated pH values using the program PropKa34 and are presented in Table 1. It should be noted here that the pK\(_a\) values are to some extent structure dependent (due to presence of salt bridges, backbone-side chain H-bonds, electrostatic repulsion or attraction and local desolvation) so that for acidic and basic pH the overall charge may vary by up to one unit. Here, we employed values which exactly agree with the experimentally observed ones.

To improve sampling, two simulations with very different initial conditions were carried out for each value of pH, with one trajectory started with the \( \beta \)-amyloid placed at the air–water interface and other in the middle of the slab. The total time of each trajectory was 230 ns, with the information about the initial position of the \( \beta \)-amyloid being lost within the first few tens of nanoseconds. From these, the first 50 ns were discarded when density profiles were calculated, i.e., averaged distributions of all the atoms across the slab. The employed time step was 1 fs and coordinates were saved every 1 ps. By performing block averages, we checked that the simulation length was sufficient to provide converged structural results in terms of radius of gyration of the \( \beta \)-amyloid.

We employed the parm99 force field for the polypeptide,35 the SPC/E water model,36 together with (nonpolarizable) parameters for Cl\(^-\) and K\(^+\) ions,35 needed to compensate the net charge of the experiment, acidic (pH = 3), neutral (pH = 7), and basic (pH = 10) solutions used. PBS buffer pH 7.4 (---) and PBS buffer pH 7.4 with \( \beta \)-amyloid 1–16 (---) are shown in blue, which establishes the wavelengths at which the \( \beta \)-amyloid 1–16 absorbs. Spectra of the salts, sodium citrate, monopotassium carbonate, dipotassium carbonate, and monosodium phosphate, used to make the different pH buffers are shown with dashed lines (---); the same salts with \( \beta \)-amyloid 1–16 present are shown with solid lines (---). See legend for absorption spectral assignments.

### COMPUTATIONAL METHODS

MD simulations were performed to investigate computationally the surface activity of the \( \beta \)-amyloid as a function of pH. As in the experiment, acidic (pH = 3), neutral (pH = 7), and basic (pH = 10) conditions were simulated. In the \( \beta \)-amyloid (i.e., polypeptide \( \text{H}_2\text{N-DAEFRHDSGYEVHHQK-COOH} \)) the titratable groups (i.e., K, R, H, D, E, Y, and the terminal groups) were either charged or neutral according to apparent pK\(_a\) values.33 The protonation states were established for each of the investigated pH values using the program PropKa34 and are presented in Table 1. It should be noted here that the pK\(_a\) values are to some extent structure dependent (due to presence of salt bridges, backbone-side chain H-bonds, electrostatic repulsion or attraction and local desolvation) so that for acidic and basic pH the overall charge may vary by up to one unit. Here, we employed values which exactly agree with the experimentally observed ones.

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β-amyloid. For the nonstandard amino acid residues charges were evaluated using the ab initio RESP procedure as recommended for the Amber force fields. The size of the unit cell was 65 Å. 3D periodic boundary conditions were applied with long-range electrostatic interactions beyond the nonbonded 9 Å cutoff accounted for using the particle mesh Ewald (PME) method. The Berendsen temperature (300 K) coupling was employed, and all bonds containing hydrogens were constrained using the SHAKE algorithm. All MD simulations were performed using the Amber 10 program.

**RESULTS AND DISCUSSION**

**Experimental Section.** β-Amyloid (1–16) has UV absorption maxima at both 220 nm, due to the peptide backbone, and at 280 nm due to the amino acid side chains (Figure 1). For the second harmonic generation (SHG) study, the backbone absorption was exploited because it is much stronger, and the available laser power is higher and more stable at (pump wavelengths) 400 nm than near 560 nm. Although the phosphate ions in the buffer begin to absorb at 200 nm, those absorptions are much weaker than that of the amyloid at 200 nm (Figure 1), and the buffer is kept at a constant concentration throughout the experiment, thereby producing a constant background SHG signal. Changes in the SHG intensity with bulk concentration and/or time are therefore attributed to amyloid absorption to the air–water interface. The SHG response from the peptide is normalized to the background signal from the buffer so that all graphs comprise...
Here $\Delta G_{\text{abs}}$ is the Gibbs free energy of adsorption of the peptide to the interface, $C$ is the bulk peptide concentration, $C_w$ is the bulk water concentration, $N_s^{\text{max}}$ is the maximum surface concentration, and $N_s$ is the surface concentration of the peptide. The SHG response was fit to the Langmuir model, yielding a Gibbs free energy of adsorption of $-10.3 \text{ kcal/mol}$ at both pH 7.4 and pH 3 (Figure 2). The reported uncertainty is one standard deviation from a fit of the data, weighted by reciprocal variances, but the actual uncertainty is certainly large, primarily due to the model dependence. An attempt was made to fit the data to the amphiphilic isotherm model, which incorporates additional parameters describing aggregation of monomers and the interaction of charged particles, but no significant dependence was found for those parameters. Hence, the simple Langmuir model was considered sufficient for determining the free energy for the peptide adsorbing to the air–water interface. We estimate a 30% uncertainty in our determination of this quantity.

Within our experimental precision, the free energy of adsorption for $\beta$-amyloid 1–16 is not significantly different between an acidic solution (pH 3) or neutral solution (pH 7), as shown in Figure 2, but the peptide exhibits no detectable presence at the interface in a basic solution (pH 10) at any concentration. All measurements, for determining the free energy, were taken 30 min after solution preparation, well before any peptide aggregation processes occur.

The buffer ions are not responsible for the partitioning of the $\beta$-amyloid 1–16 to the air–water interface. The SHG response of the peptide was measured for water without any buffer, using strong acid and base to reach pH 3 and 11, respectively, and the same response was found as for the buffered solutions (Figure 2).

At pH 7, the $\beta$-amyloid 1–16 has four negatively charged side chains and two positively charged side chains. At pH 3 there are five positively charged side chains and one that is negatively charged, whereas at pH 10 there are five negatively charged side chains and one positively charged side chain. It is probable that the charge balance of the peptide strongly affects its surface affinity, as the hydration properties of the residues comprising

Figure 7. Number density profiles from MD simulations for all heavy atoms of $\beta$-amyloid at pH = 3, 7, and 10, symmetrized with respect to the middle of the slab and averaged over two initial conditions. The $\beta$-amyloid profile is in red, and the profile of water is in blue. GDS stands for the Gibbs dividing surface.
the side chains vary considerably, and these are likely to be determining factors.

There appear to be two different time scales for β-amyloid 1–16 adsorption to the air–water interface. The shortest of these describes the absorption of the peptide to the interface (Figure 3), which occurs over the course of minutes. The maximum surface concentration is reached after 30 min for 20 μM β-amyloid 1–16, wherein the SHG signal levels off. On this shorter time scale, equilibrium between the bulk and interfacial peptide concentrations appears to have been reached. This SHG-measured time dependence for the absorption of β-amyloid 1–16 is similar to the time scale previously found for the adsorption of the full length β-amyloid protein, as determined by changes in the surface tension.7

On a longer time scale, the SHG response exhibited further changes (Figure 4). The SHG signal decreased over the course of hours, depending on the bulk concentration of the β-amyloid 1–16. For these experiments, the first measurement was taken 30 min after the solution was mixed. For 20 μM β-amyloid 1–16, the maximum SHG response was already reached at t = 0 and the SHG response decreased with time, reaching a minimum (Figure 4). Upon decreasing the β-amyloid 1–16 concentration to 10 μM, the t = 0 SHG response began increasing and did not reach a maximum until 1 h, whereupon it then decreased until it matched the SHG response of the 20 μM solution. For the 2.5 μM solution, the maximum SHG response occurred after 2 h, decreasing thereafter to the same constant value as for the 20 and 10 μM solutions. The 1 μM solution showed the same trend, except that it exhibited its maximum SHG signal after 4 h, at the same level to which the higher concentration solutions had stabilized (Figure 4). No evidence of optical damage was observed in the present measurements.

The faster time scale found here (Figure 3) can be related to saturation of the surface with peptide, because the BAM experiments described below yield a correlating result. At higher bulk concentrations, it takes less time for the air–water interface of the solution to become saturated with peptide, which is...
evidenced by observing the maximum SHG response on a faster time scale. The β-amyloid 1–16 comprises only 16 amino acids, which can form an α helix. The decrease in SHG response is likely due to the rearrangement of the β-amyloid 1–16 at the interface; i.e., once the surface is saturated with the peptide, it rearranges.9 We speculate that this rearrangement could involve an unfolding and refolding of the peptide, thereby producing a greater SHG response, since the alpha helical structure is more ordered than the unfolded structure. This contention is further supported by our BAM experiments described below. Using Brewster angle microscopy, the saturation of the surface and rearrangement, or entanglement, of the β-amyloid 1–16 peptide was observed directly. A series of images was collected every one to two minutes after the water surface (the air-water interface) was refreshed by aspiration. A representative data set is shown in Figure 5. The lighter color in the images is due to water and the darker colored objects in the images are the peptide domains. Initially (at time zero), there is very little peptide at the interface, but over time, more appears. After 5 and 8 min, the β-amyloid 1–16 has formed entangled structures at the interface that appear stretched out. As additional peptide adsorbs to the surface, the entangled structure continues to get denser, until after around 25 min, bare water surface is no longer visible. Unlike previous studies of proteins at the air-water interface,48 we do not observe a homogeneous distribution of the β-amyloid 1–16 peptide over the surface. Rather, as the peptide absorbs to the interface, it appears to form entangled domains, as described above. Due to the resolution of the microscope and the size of the peptide, the strands seen in the images cannot be due to single β-amyloid 1–16 peptides but must result from a number of interacting peptide strands. This domain formation is also visible via SHG in terms of large fluctuations in signal that correspond to domains moving across the surface optical spot (Figure 6a,b). Distinct bursts of SHG response occur from the interfacial peptide at all concentrations. Due to air flow, the surface is not static; the peptides drift in and out of the excitation region. The fluctuations observed during the measurement of the SHG response for the buffers are a measure of the intrinsic noise of the system (Figure 6c), which is at least an order of magnitude lower than the fluctuations in SHG response attributed to the domain formation of the peptide at the air-water interface.

Figure 10. Same as Figure 9, but for pH = 10.

Figure 11. Time evolution of number density profile of all heavy atoms of individual amino acids of β-amyloid at pH = 10, initially placed in interfacial region. Note the largest surface affinity of phenylalanine (when the peptide is at the interface).
Recent work by Triulzi et al.\(^1\) demonstrates a presence of \(\beta\)-amyloid 25–35 at the air-water interface, where it was found to aggregate. Here, we demonstrate that \(\beta\)-amyloid 1–16, i.e., the protein amino acid sequence that induces aggregation, not only exists at the air-water interface but also actually exhibits a strong preference for the interface relative to the bulk. \(\beta\)-Amyloid 1–16 does not form distinct, observable fibrils, but it does aggregate into domains (Figure 5). These domains created by the 1–16 section of \(\beta\)-amyloid may assist fibril formation by bringing the 25–35 sections of \(\beta\)-amyloid into close contact.

**Computational Details.** The simulated trajectories were first analyzed in terms of density profiles of all the heavy atoms, i.e., averaged distributions in the \(z\)-direction from the center of the slab to the water—vapor interface. Density profiles of heavy atoms of the \(\beta\)-amyloid and water, evaluated for the three investigated pH values of 3, 7, and 10 are shown in Figure 7. We see that at pH 7 the \(\beta\)-amyloid strongly segregates to the water surface. In contrast, at pH 3 and 10 the polypeptide distributes almost evenly across the slab. At pH 7 the absolute value of the charge on the \(\beta\)-amyloid is the lowest amounting to \(2e\), while at both pH 3 and 10 the absolute values of the overall charge is \(5e\) (see Table 1). We thus observe an anticorrelation between the absolute value of the charge and the surface propensity of the \(\beta\)-amyloid. This can be rationalized using simple Coulombic arguments: the larger the total charge, the stronger the hydration (i.e., the weaker the surface affinity) of the peptide.

While averaged values are presented in Figure 7, time evolutions of density profiles are shown together with the time evolution of radii of gyration of the \(\beta\)-amyloid at pH 3, 7, and 10 in Figures 8, 9, and 10. These plots carry detailed information about position, orientation, and compactness of \(\beta\)-amyloid during the simulations. The width of the density profile tells us to which extent the \(\beta\)-amyloid is extended along the \(z\)-axis and what is its approximate orientation with respect to the interface (i.e., parallel or perpendicular). The radius of gyration allows us to additionally distinguish between compact and unfolded structures. The main message from this analysis is that the size of the peptide does not change significantly when exposed at the interface compared to the situation in the aqueous bulk.

We also plotted the \(z\)-profiles at different pHs for individual amino acids (which were grouped with respect to their polarity, i.e., hydrophobic, polar, and charged residues). Due to the large number of polar and charged residues, the surface affinity of the hydrophobic residues was reduced by the presence of their polar or even charged environment. The most surface active amino acid was found to be phenylalanine, as shown in Figure 11. PHE was consistently the residue lying closest to the interface, once the \(\beta\)-amyloid reached the surface region. Other, less hydrophobic amino acids (glycine, alanine, and valine) did not exhibit such a strong surface exposure. However, as the pH was varied, aromatic residues (TYR and HIS) showed, when uncharged, surface exposure similar to PHE. However, this surface affinity disappeared once these amino acids became charged. The same behavior was also apparent from calculations of solvation properties of hydrophobic residues, by evaluating either the solvent accessible surface (SAS) or the number of water molecules around each residue.

## CONCLUSIONS

In conclusion, our SHG experiments have shown that \(\beta\)-amyloid 1–16 exhibits a large free energy of interfacial adsorption of \(-10.3\) kcal/mol at both acidic and neutral bulk pH, but interestingly, it exhibits no presence at the air—water interface in a strongly basic solution. The peptide forms entangled domains and strands at the interface, but no \(\beta\)-amyloid fibril formation was observed. The \(\beta\)-amyloid 1–16 peptide exhibits absorption and reorganization at the interface on very different time scales. It takes longer than our experimental time to actually generate fibrils, \(^5\) but the domains formed could comprise nucleation sites for subsequent fibril formation. The strong pH dependence of surface behavior of the \(\beta\)-amyloid was also confirmed by MD simulations, which show that it anticorrelates with the overall charge of the peptide. The largest surface affinity was observed at pH = 7, with little surface exposure at pH 10 and 3. The first two findings are in agreement with the SHG experiment; however, simulations do not support strong surface affinity in the third case. It is possible that increased interfacial ordering of the peptide occurs at low pH values, which would also enhance the SHG transition strengths, and this effect is not captured in the MD simulations.
(46) Petersen, P. B.; Saykally, R. J. Manuscript in preparation.