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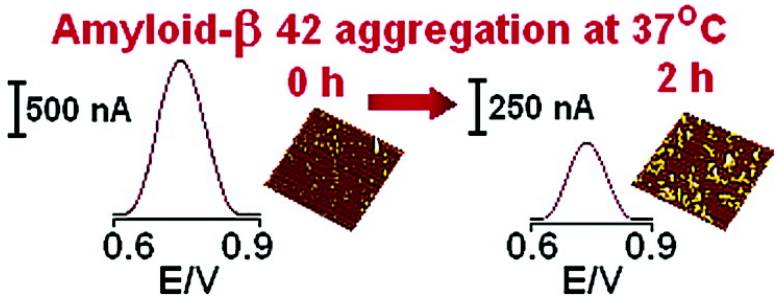

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A Rapid Label-Free Electrochemical Detection and Kinetic Study of Alzheimer's Amyloid Beta Aggregation

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We present the first electrochemical detection, characterization, and kinetic study of the aggregation of Alzheimer's disease (AD) amyloid beta peptides (Aβ-40, Aβ-42) using three different voltammetric techniques at a glassy carbon electrode (GCE). This method is based on detecting changes in the oxidation signal of tyrosine (Tyr) residue. As the peptides aggregate, there are structure conformational changes, which affect the degree of exposure of Tyr to the molecular surface of the peptides. The results show significant differences in the aggregation process between the two peptides, and these correlate highly with established techniques. The method is rapid and label-free, and the principle can be universally applied to other protein aggregation studies related to diseases, such as Huntington's, Parkinson's, and Creutzfeldt Jacob (CJD). This method could also be explored in screening for the effectiveness of AD therapies.

One of the hallmarks of AD is the formation of neuritic plaques in the brain of AD individuals. The aggregation of amyloid beta (Aβ) peptides is central to the formation of the plaques. Aβ is a 4 kDa peptide present in the brain and cerebral spinal fluid. In its native form, Aβ is unfolded but aggregates into a β-sheet structure of ordered fibrils under various conditions.[1−3] Aβ-42 is more hydrophobic and aggregates more easily than Aβ-40 and is predominant in the plaques of AD individuals.[4,5] The aggregation process starts with a nucleation step followed by a growth phase, which is dependent on the composition of the carboxyl end of Aβ.[6]

Aβ aggregation, in vitro, is commonly studied using techniques such as CD spectroscopy, fluorescence detection, and electron and atomic force microscopy (AFM).[2,7,8] In particular, the detection of Aβ aggregation and the visualization of these plaques using Thioflavin T (Th-T) and its derivatives has become a reference method.[9−10] Th-T binds in a specific, regular fashion to amyloid fibrils. Confocal microscopy studies performed by Krebs and co-workers[11] revealed that Th-T could bind to amyloid fibrils such that their long axes were parallel. Proteins have been detected by electrochemical oxidation of Tyr, tryptophan (Trp), and cystine/cysteine residues in assays sometimes involving adsorption/desorption phenomena.[12−15] The first introduction of the direct oxidation of Trp and Tyr residues on carbon electrodes was reported about two decades ago.[16,17] Oxidation of Tyr and Trp at a wax-impregnated spectroscopic graphic electrode (WISGE) is reported to be a two-electron-transfer process.[18] Although Aβ possesses only one redox-active residue, Tyr, at position 10, we assumed that the changes in conformation and possibly charge(s) due to nucleation and later aggregation of the peptide might affect the adsorbability of the Tyr residue to the electrode surface, thus enabling us to detect the aggregation process and possibly the initial stages. Moreover, since the method is based on conformational structure change, the study could provide information regarding the different structures that the peptides adopt prior to and during the aggregation process. Our electrochemical results have also been compared with the fluorescence results obtained by using Th-T as the aggregation indicating probe.

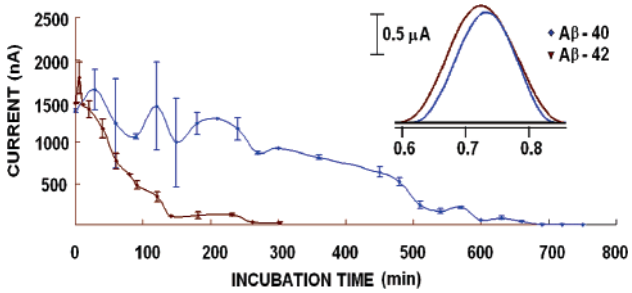

Figure 1. Kinetic study of Aβ-42 (red line) and Aβ-40 (blue line) aggregation after incubation at 80 μM in TBS at 37 ± 1 °C; detected at 8 and 4 μM, respectively, using SWV, at room temperature. Inset: Voltammograms of native Aβ-42 (red line) and Aβ-40 (blue line); detected at 8 and 4 μM, respectively, using SWV, at room temperature.

Cyclic voltammetry of native forms of the Aβ-peptides was carried out. The oxidation of Tyr in a 20 mM Tris/HCl buffer, pH 7.0 (TBS), was found to be irreversible, in agreement with previous reports.[19,20] The peptides adsorbed on the electrode surface; the final oxidation product was not electroactive and blocked the electrode surface. As the scan rate (υ) was increased from 25 to 200 mV/s, there was less time for the diffusion layer to extend into the bulk of the solution, causing a large diffusion gradient and, consequently, a higher peak current.[21] The peak current was proportional to the square root of υ. We, therefore, concluded that the electrode response was controlled by semi-finite diffusion. The diffusion coefficients of the Aβ-42 and Aβ-40 across the electrode surface were estimated at $2.1 \times 10^{-7}/\alpha$ and $2.03 \times 10^{-7}/\alpha$ cm^2/s, respectively, according to the Randles−Sevcik equation.[22] Differential pulse voltammetric (DPV) and CV studies showed that the oxidation of both peptides involved the transfer of two electrons.[21] An almost linear relationship ($r^2 = 0.9918$) between the peak potential and log υ further confirmed the $2e^-$ transfer process and irreversibility of the oxidation process.

The peak potential of the two peptides was very similar (inset of Figure 1). The dependence of detected current signal on the concentration of the analyte was studied using square wave voltammetry (SWV), and the results showed that an increase in concentration led to an increase in peak current, but the relationship was nonlinear. Concentration was increased until there was no increase in peak current (saturation) or until there was a change in peak shape (surface fouling). For $n = 3$, the relative standard deviation (RSD) of peak potential ranged from 0.3 to 1.4% and from 0.4 to 3.0% for Aβ-40 and Aβ-42, respectively. The RSD for the current signal ranged from 1.2 to 8.3% for Aβ-40 and from 1.5 to 8.6% for Aβ-42. The detection limits, estimated from S/N = 3, corresponded to approximately 0.7 μg/mL for both peptides.

The aggregation kinetics were analyzed after incubation of the peptides at 80 μM in TBS at 37 ± 1 °C using SWV (Figure 1).

Samples were analyzed until the current signals of the peptides were indistinguishable from background noise, that is, after 300 and 750 min incubation period for Aβ-42 and Aβ-40, respectively. The peptides (80 μM) were also analyzed using a spectrofluorometer

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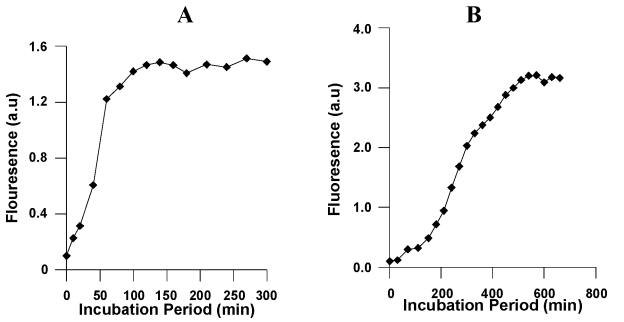

Figure 2. Kinetic study of Aβ-42 (A) and Aβ-40 (B) aggregation after incubation at 80 μM in TBS at 37 $\pm$ 1 °C; detected using Th-T fluorescence dye. The same arbitrary units (a.u.) are used throughout this report.

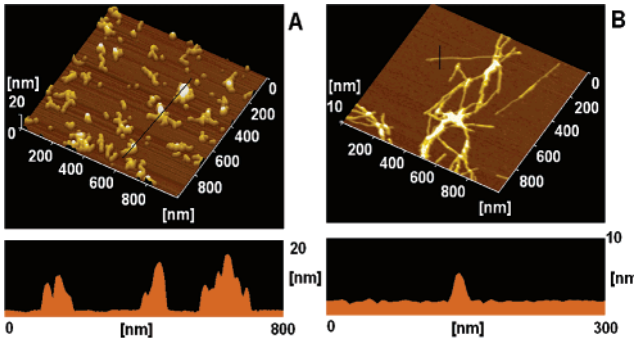

Figure 3. AFM images of Aβ-42 aggregates after incubation at 80 μM in TBS at 37 $\pm$ 1 °C for 120 min on bare mica surface (A) and for 180 min on APTES-modified mica surface (B).

in conjunction with Th-T as the indicating probe at excitation and emission wavelengths of 450 and 490 nm, respectively. The electrochemical data correlate highly ($r = -0.9022$ and -0.9385 for Aβ-42 and Aβ-40, respectively) with that obtained using Th-T fluorescence detection (Figure 2). After incubation at specified time periods, Aβ-peptides were deposited on a bare mica disk surface and on a 3-(aminopropyl)triethoxysilane (APTES)-modified disk surface. After, the disk was rinsed with purified water and dried using nitrogen gas. The contact angles were measured at 2 and 36° for the bare mica surface and the APTES-modified mica surface, respectively. AFM images (Figure 3) were obtained in air in a dynamic force mode at optimal force.

The AFM results for Aβ-42 support well the electrochemical and fluorescent results. However, the AFM images of Aβ-40 showed hardly any change in the aggregation state of the peptide over time. We assume that the affinity of the peptide to the mica surface was very low.

Further, a comparison of the kinetic data of Aβ-40 and Aβ-42 shows that Aβ-40 adopts more varied conformational structures compared to Aβ-42, as seen by the fluctuations of the Tyr signal displayed by Aβ-40. The difference might be attributed to the distinct oligomerization pathways observed for the two peptides during the early stages of aggregation,[21] thus making the electrochemical method slightly more informative than the labeled technique. The presence of metal ions, in particular, copper, zinc, and iron, has been reported to enhance Aβ aggregation.[24,25] Further, copper has been reported to mediate dityrosine cross-linking in Aβ-peptides.[26] None of these metals were detected in our buffer by graphite furnace atomic absorption spectroscopy (GFAAS). Trace amounts of iron (3.15, 35.2 μM) and zinc (0.26, 3.9 μM) were detected in Aβ-40 and Aβ-42 samples, respectively. Copper ions were not detected. The presence of iron and zinc would possibly influence the rate of peptide aggregation[24,25] and probably have some effect on the Tyr oxidation signal. Their effect, if any, is being evaluated and will be reported in a separate submission. Bovine serum albumin, used as a control in these studies, displayed no changes in either the fluorescence signal (agreeing with a previous report[26]) or the Tyr oxidation signal. Also, the aggregation kinetics of Aβ-42 incubated at 0 $\pm$ 1 °C showed no changes in the Tyr signal, not surprising since hydrophobic interactions are destabilized by low temperatures,[27] further confirming the validity of our method for detecting the aggregation of Aβ.

In summary, we report the first bioelectrochemical study of Aβ-peptides using various voltammetric techniques at a GCE. The kinetics of the peptide aggregation have been studied also for the first time using electrochemistry. The method compares favorably with established optical techniques and offers some advantages over these methods. Furthermore, it is rapid and direct (label-free), and the principle can be universally and readily extended to other protein aggregation studies. The method also has potential as a drug-screening tool and/or for assessing, in vitro, the effectiveness of AD therapeutics that target Aβ plaques.

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Supporting Information Available: Related instrumentation, procedures and graphics for the optimization studies (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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