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Electrochemical Oxidation of Benzothiazole Dyes for Monitoring Amyloid Formation Related to the Alzheimer's Disease

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Alzheimer's disease (AD) is associated with the formation and deposition of amyloid fibrils. A better understanding of the oligomeric intermediates on the pathway to fibrilization is highly desired, but efficient methods for their detection are lacking. We have studied the interfacial properties of amyloid peptides (Aβ-40 and Aβ-42) and the course of their aggregation in vitro in the presence of the benzothiazole dyes Thioflavin T (4-(3,6-dimethyl-1,3-benzothiazol-3-ium-2-yl)-*N*,*N*-dimethylaniline) chloride, ThT) and BTA-1 ([2-(4′-(methylamino)phenyl) benzothiazole]) using electrochemical techniques. The intercalative properties of these dyes between the β-sheets of amyloids have been well-documented using fluorescence-based systems, but their electrochemistry is reported here for the first time. ThT is positively charged and water-soluble, whereas BTA-1 is neutral and hydrophobic. Immediate and significantly different electrochemical characteristics of these dyes were observed in the presence of amyloid peptides. A decrease of the BTA-1 oxidation signal was observed upon incubation with Aβ-40. Incubation of BTA-1 with Aβ-42 results in an increased rate of exponential decay, which was in agreement with the known rapid aggregation properties of Aβ-42. The aggregation of amyloid peptides with ThT resulted in an unexpected increase in signal after 24 h of incubation, consistent for both peptides. The results of the electrochemical trials were confirmed using simultaneous fluorescence analysis of the same incubated amyloid samples. The very early changes in the interfacial behavior of the amyloid peptides after the first few minutes of incubation were attributed to the fast oligomerization of the peptides with the disruption of the intercalative properties of the benzothiazole dyes between the β-sheets. The subsequent changes in the electrochemical signals can be related to the onset of intercalation between the fibrils. Our results demonstrate the utility of electrochemical oxidation signals of the benzothiazole dyes as a new and simple tool for the investigation of amyloid formation related to the AD.

Alzheimer's disease (AD) is a neurodegenerative disease marked by clinical symptoms such as a decline in cognitive ability, alterations in behavior, irreversible memory loss, and language impairment.[1] The onset of the disease generally occurs over the age of 60 and is not considered a natural pathway of aging.[2] AD is currently estimated to afflict more than 5 million Americans, a value that is expected to triple over the next 50 years.[1]

AD is one of numerous neurodegenerative diseases including Parkinson's, Huntington's, and Creutzfeld Jacob disease in which symptom progression is correlated to the development of neural fibrillary tangles (NFT) and neuritic plaques deposited within the limbic and association cortices of the brain.[3] Though the sequence of events in plaque formation is not well-defined, it is understood that the development is related to the hyperphosphorylation of the tau protein,[4] a structural component present in microtubule polymerization. The increased hyperphosphorylation of tau protein is attributed to the aggregation of a specific group of peptides referred to amyloid peptides.[5] Amyloid is the generic term describing abnormally fibrillated proteins possessing a β-pleated sheet conformation.[6] The focus of this report is to monitor the peptide aggregation specific to AD, as it is a model class for general amyloid aggregation of similar neurodegenerative diseases.[7] The β-peptides are also the most well-characterized subdivision of amyloid peptides, allowing us to confirm and compare our findings with those of previous studies.[7]

Due to the dynamic structural changes to Aβ during aggregation, conventional methods used to determine structure, such as X-ray crystallography and solid state NMR spectroscopy, are less effective in monitoring the aggregation process. Instead, spectroscopic techniques including infrared and vibrational circular dichromism are employed, along with other techniques such as atomic force microscopy, fluoresence spectroscopy, and electrochemistry to monitor the amyloid aggregation.[8−17] Especially, electrochemical techniques have

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(1) http://www.alz.org/alzheimers_disease_alzheimer_statistics.asp.
(2) Rauk, A. *Chem. Soc. Rev.* **2009**, *38*, 2698–2715.
(3) Jellinger, K. A. *J. Neural Transm.* **2006**, *113*, 1603–1623.
(4) Hanger, D. P.; Anderton, B. H.; Noble, W. *Trends Mol. Med.* **2009**, *15*, 112–119.
(5) Delacourte, A.; Sergeant, N.; Buee, L. *Brain Aging* **2002**, *2*, 16–17.
(6) Shapira, R.; Austin, G. E.; Mirra, S. S. *J. Neurochem.* **1988**, *50*, 69–74.
(7) Wu, C.; Wang, Z.; Lei, H.; Duan, Y.; Bowers, M. T.; Shea, J. E. *J. Mol. Biol.* **2008**, *384*, 718–729.
(8) Szabo, Z.; Klement, E.; Jost, K.; Zarandi, M.; Soos, K.; Penke, B. *Biochem. Biophys. Res. Commun.* **1999**, *265*, 297–300.

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been shown to be very effective for monitoring the early stages of the amyloid formation.[13−17]

Since Aβ peptides possess low intrinsic fluorescence properties (only a single Tyr residue), staining with fluorescent dyes such as Congo Red and Thioflavin T (4-(3,6-dimethyl-1,3-benzothiazol-3-ium-2-yl)-*N*,*N*-dimethylaniline) chloride, ThT) is required.[18,19] The excitation wavelength of ThT is approximately 440−450 nm. In the presence of amyloid fibrils, ThT undergoes a 115 nm red shift in excitation spectrum producing a fluorescence emission occurring from 475 to 600 nm.[19] This shift does not occur in the presence of amyloid peptide monomers.[7] The fluorescent induction by Aβ aggregate binding is thought to occur by an alteration in chemical environment by hydrogen bonding at the β-sheets of the fibril.[20] Binding occurs along the axis of the strand, parallel to the elongation direction.[21] The binding points of ThT and derivatives, such as BTA-1 ([2-(4′-(methylamino)phenyl) benzothiazole]), occur at within the β-pleated sheets of Aβ at three specific points: side β-sheets (Type Ia), central β-sheets (Type Ib), and end β-sheets (Type II).[7] BTA-1, a neutral analogue of ThT, is produced through the removal of the methyl group from nitrogen within the heterocyclic unit of ThT.[22] The fluorescent properties of BTA-1 are comparable to that of ThT.[22] The affinity of BTA-1 (K_i = 20.2 nM) relative to the affinity of ThT (K_i = 890 nM) for Aβ-40 fibrils is significantly greater.[7,23] Moreover, the greater degree of lipophilicity expressed by BTA-1 relative to ThT allows for it to cross the blood-brain barrier, making it a more suitable candidate to monitor Aβ aggregations within the brain in vivo.[24]

To the best of our knowledge, we are reporting the changes observed in the electrochemistry of the benzothiazole dyes ThT and BTA-1 upon in vitro interaction with Aβ peptides for the first time. Our aim was to monitor the aggregation process of Aβ-40 and Aβ-42 by electrochemical analysis of the changes in the oxidation signals of ThT and BTA-1 on carbon electrodes. Confirmation of the aggregation reactions during incubation was achieved through the well-established fluorescence experiments, the results of which were in agreement with the previously reported studies.[8−12]

EXPERIMENTAL SECTION

Reagents. Amyloid-beta (Aβ) peptides (Aβ-40, Aβ42; trifluoroacetate salt) and BTA-1 ([2-(4′-(methylamino)phenyl) benzothiazole]) were purchased from EMD Chemicals Inc. (Gibbstown, NJ). Thioflavin T (ThT, 4-(3,6-dimethyl-1,3-benzothiazol-3-ium-2-yl)-*N*,*N*-dimethylaniline chloride) was purchased from Sigma-Aldrich (Oakville, ON). Unless otherwise stated, all other reagents were of analytical grade and used as received. Ultrapure water, obtained from Cascada LS (Pall Co., NY) water purification system at 18.2 MΩ, was used in the preparation of buffers and cleaning of the spectrofluorometer analysis stage.

Apparatus. Electrochemical analyses were performed by means of differential pulse voltammetry (DPV) and cyclic voltammetry (CV) using a μAutolab-III electrochemical analysis system (Eco Chemie, Kanaawleg, The Netherlands) operated in conjunction with its general-purpose electrochemistry software (GPES). The planar screen-printed carbon strip (SPCS) electrodes were kindly donated by Professor Eiichi Tamiya (Osaka University, Japan) and Biodevice Technology Ltd. (Kanazawa, Japan). These strips consisted of a carbon working electrode (geometric working area: 2.64 mm^2), a carbon counter-electrode, and a Ag/AgCl reference electrode. Electrochemical deposition and measurements were performed in 20 μL of the sample solution covering all three electrodes in the horizontal position. All measurements were taken at room temperature (24 ± 1 °C). A Nanodrop 3300 spectrofluorometer (Thermo Scientific, Wilmington, DE) was used to detect the fluorescence signal of native and amyloid-bound ThT and BTA-1. Samples were measured in 2 μL aliquots.

Procedure. BTA-1 stock solution (20.8 mM) was prepared with dimethyl sulfoxide (DMSO) and stored at 20 ± 1 °C. ThT stock solutions (10 mM) were prepared daily with ultrapure water and stored at room temperature. Both BTA-1 and ThT stock solutions were stored in aluminum foil covered tubes to avoid photodegradation. Aβ-40 stock solution (534.8 μM) and Aβ-42 stock solution (553.7 μM) were prepared from 50 mM (PBS), pH 7.4, and stored at −20 ± 1 °C to prevent aggregation. Prior to analysis, sample vials were allowed to equilibrate to RT. Spontaneous aggregation of peptide samples (50 μM) was induced through incubation with BTA-1 or ThT (50 μM) at 37 ± 1 °C using a thermal block-shaker (Fisher Scientific).

Sample Preparation. ThT was readily water soluble, but BTA-1 stock solution was prepared in DMSO. Both dyes were further diluted to the desired concentrations using 50 mM phosphate buffer solution (PBS, pH 7.4). DPV and fluorescence measurements were performed for each concentration. These procedures were repeated for BTA-1. CV measurements were taken for 1 mM samples of ThT and BTA-1, individually, at various scan rates. Peptide samples were prepared with a mixture of 50 μM of the benzothiazole dye with 50 μM amyloid peptide in PBS. Samples were kept in a PCR vial (200 μL total volume) with a tightly closed lid to avoid evaporation. The vials were placed in a thermo-block shaker, which was preheated

(9) Ono, K.; Yoshiike, Y.; Takashima, A.; Hasewaga, K.; Naiki, H.; Yamada, M. *J. Neurochem.* **2003**, *87*, 172–181.
(10) Yoshiike, Y.; Tanemura, K.; Murayama, O.; Akagi, T.; Murayama, M.; Sata, S.; Sun, X.; Tanaka, N.; Takashima, A. *J. Biol. Chem.* **2001**, *276*, 32293–32299.
(11) Klunk, W. E.; Wang, Y.; Huang, G. F.; Debnath, M. L.; Holt, D. P.; Mathis, C. A. *Life Sci.* **2001**, *69*, 1471–1484.
(12) Klunk, W. E.; Wang, Y.; Huang, G.; Debnath, M. L.; Holt, D. P.; Shao, L.; Hamilton, R. L.; Ikonomovic, M. D.; DeKosky, S. T.; Mathis, C. A. *J. Neurosci.* **2003**, *23*, 2086–2092.
(13) Vestergaard, M.; Kerman, K.; Saito, M.; Nagatani, N.; Takamura, Y.; Tamiya, E. *J. Am. Chem. Soc.* **2005**, *27*, 11892–11893.
(14) Vestergaard, M.; Kerman, K.; Tamiya, E. *NanoBiotechnology* **2006**, *2*, 5–16.
(15) Chikae, M.; Fukuda, T.; Kerman, K.; Idegami, K.; Miura, Y.; Tamiya, E. *Bioelectrochemistry* **2008**, *74*, 118–123.
(16) Palecek, E.; Ostatna, V.; Masarik, M.; Bertoncini, C. W.; Jovin, T. M. *Analyst* **2008**, *133*, 76–84.
(17) Vestergaard, M.; Kerman, K. *Curr. Pharm. Anal.* **2009**, *5*, 229–245.
(18) Levine, H. *Amyloid* **2005**, *12*, 5–14.
(19) Fodera, V.; Groenning, M.; Vetri, V.; Librizzi, F.; Spagnolo, S.; Cornett, C.; Olsen, L.; Van de Weert, M.; Leone, M. *J. Phys. Chem. B* **2008**, *112*, 15174–15181.
(20) Khurana, R.; Coleman, C.; Ionescu-Zanetti, C.; Carter, S. A.; Krishna, V.; Grover, R. K.; Roy, R.; Singh, S. *J. Struct. Biol.* **2005**, *151*, 229–238.
(21) Krebs, M. R. H.; Bromley, E. H. C.; Donald, A. M. *J. Struct. Biol.* **2005**, *149*, 30–37.
(22) Mathis, C. A.; Bacskai, B. J.; Kajdasz, S. T.; McLellan, M. E.; Frosch, M. P.; Hyman, B. T.; Holt, D. P.; Wang, Y.; Huang, G.-F.; Debnath, M. L.; Klunk, W. E. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 295–298.
(23) Rodriguez, C.; Sanchez de Groot, N.; Rimola, A.; Alvarez-Larena, A.; Lloveras, V.; Vidal-Goncedo, J.; Ventura, S.; Vendrell, J.; Sodupe, M.; Gonzalez-Duarte, P. *J. Am. Chem. Soc.* **2009**, *131*, 1436–1451.
(24) Mathis, C. A.; Wang, Y. M.; Holt, D. P.; Huang, G. F.; Debnath, M. L.; Klunk, W. E. *J. Med. Chem.* **2003**, *46*, 2740–2754.

to 37 ± 1 °C with a constant shaking of 200 rpm. The thermoblock shaker was also covered to avoid light exposure to the samples and the evaporation of the mixture. The aliquots (20 μL) of peptide/dye mixtures were pipetted out of the vials and dispensed on the working electrode surface of the SPCSs for electrochemical measurements and also another aliquot (2 μL) was dispensed on the detection stage of the fluorospectrometer for fluorescence measurements. These measurements were taken at desired time intervals over 24 h.

Electrochemical Detection. A 20 μL aliquot of sample was applied to the working electrode area of SPCS. Sample signals were measured from the oxidation signal of ThT or BTA-1 by DPV (applied potential, from 0 to 1 V vs Ag/AgCl within SPCS; step potential, 5 mV; amplitude, 25 mV). The raw DPV voltammograms were treated with the Savitzky-Golay level 4 smoothing feature of GPES. The baseline correction feature was set to a peak width of 0.003 V. Each electrochemical measurement condition for DPV was performed for at least three trials ($n = 3$). For CV measurements, three scans were taken simultaneously from a single 20 μL aliquot of sample. Samples were changed for each scan rate measurement: 10, 50, 100, 250, and 500 mVs^{-1}. After each measurement, the used SPCS was disposed and a new strip was employed for the consecutive measurement; thus, the nonspecific adsorption and cross-contamination problems between the electrodes could be eliminated. Error bars indicate the standard deviation of signals obtained from triplicate measurements on separate strips under similar conditions.

Fluorescence Detection. Prior to measurements, the sample stage was cleaned with 5 μL of 3 M HCl and incubated for 3 min followed by incubation with 5 μL of ultrapure water for 5 min. Fluorescence profiles for the determination of excitation and emission wavelengths were taken for 100 μM ThT and BTA-1, individually. Subsequent measurements were taken with the profile parameters set to the appropriate dye. The fluorescence intensity data were collected at an emission wavelength of 490 and 432 nm for ThT and BTA-1, respectively. Each measurement required a 2 μL aliquot of peptide/dye mixture sample, and relative fluorescence units (RFUs) were recorded. A minimum of three trials was taken for each measurement, as shown with error bars representing the standard deviation of the signals in the respective graphics.

RESULTS AND DISCUSSION

The aim of this report was the electrochemical characterization of amyloid formation in the presence of the benzothiazole dyes ThT and BTA-1. The well-described fluorescence characteristics of these dyes shed light on the interpretation of the electrochemical data. The interactions were observed via changes in the oxidation signal of the dyes rather than the conventional observation of the electro-active Tyr signal intrinsic to the Aβ peptides.[13−17] In order to be able to avoid any contributions from the oxidation signal of Tyr residues that exist in human Aβ peptides, the rat Aβ peptides, which do not contain any electro-active amino acid residues (see the Supporting Information for the complete sequences), were utilized in this report. Electrochemical measurements were supported with simultaneous fluorescence analysis, the procedures of which have been well-defined in previously reported studies.[8−12]

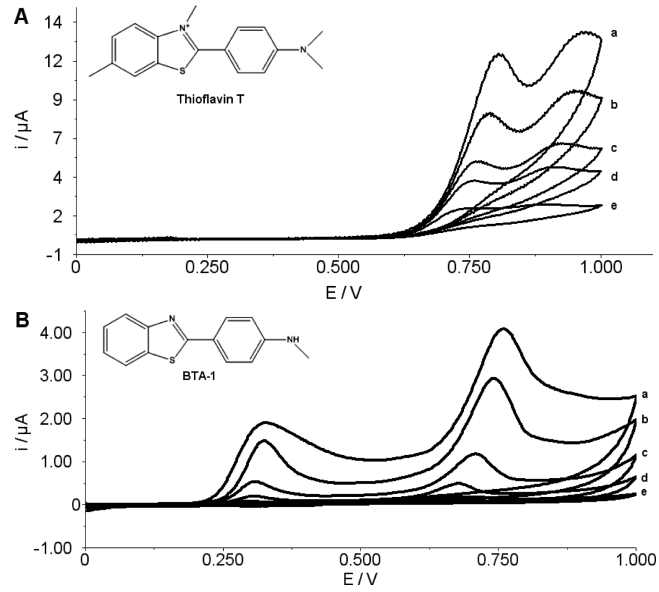

Figure 1. Cyclic voltammograms of (A) 1 mM ThT and (B) 1 mM BTA-1 at various scan rates: (a) 500 mVs^{-1}, (b) 250 mVs^{-1}, (c) 100 mVs^{-1}, (d) 50 mVs^{-1}, and 10 mVs^{-1} (e). Potential window was set from 0.0 to 1.0 V at a screen-printed carbon strip (SPCS) electrode.

Figure 1A shows changes in CV responses of 1 mM ThT at various scan rates. The peak current was found to increase linearly with the square root of scan rate (Figure 1B in the Supporting Information) indicating a diffusion-controlled electrode process.[25,26] An increase in the anodic signal for ThT was observed at approximately 590 mV for all measured scan rates. Two quasi-reversible oxidation signals occurred at 796 mV and 947 mV at 500 mVs^{-1}. These peak potentials remained approximately constant as scan rate was decreased down to 10 mVs^{-1}. No reduction signals were observed for ThT over the given potential range.

Changes in CV responses of 1 mM BTA-1 with scan rates (Figure 1B) displayed two irreversible anodic peak potentials at 330 mV and 757 mV as well as a linear increase in peak current with the square root of scan rates (Figure 2B in the Supporting Information) again indicating a diffusion-controlled electrode process.[25,26] No observable reduction signals occurred over the given potential range. The location of peak potentials of the two dyes over the given range differed significantly. In addition, ThT displayed a maximum peak current of approximately 12.6 μA, more than double that of its neutral analogue, BTA-1 (5.84 μA), at a scan rate of 500 mVs^{-1}.

The voltammograms obtained during the incubation of BTA-1 with Aβ-40 are shown in Figure 2A−C. The addition of Aβ-40 to BTA-1 resulted in an immediate decrease in the peak currents of all three BTA-1 signals (Figure 2A). The peak currents at 600 mV and 810 mV decreased immediately and remained consistently low over the 24 h incubation period (Figure 2B). Whereas the peak potential at 290 mV decreased slowly from 30 to 6 nA over 24 h. As for the control experiments, we monitored the changes

(25) Bond, A. M. *Modern Polarographic Methods in Analytical Chemistry*; Marcel Dekker: New York, 1980.
(26) Nicholson, R. S.; Shain, I. *Anal. Chem.* **1965**, *36*, 706–723.

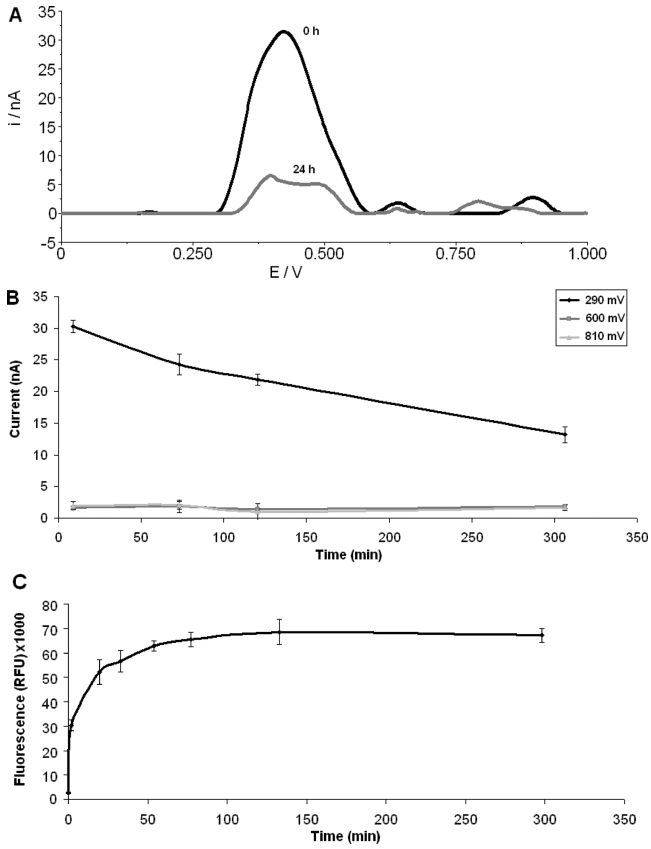

Figure 2. (A) DPV voltammograms of 50 μM BTA-1 incubation in the presence of 50 μM Aβ-40 at 37 °C in 50 mM PBS (pH 7.40) using SPCSs. Aliquots of the peptide/dye mixture were taken from the incubating samples at predetermined time intervals for the electrochemical and fluorescence measurements. Details of the measurement conditions are described in the Experimental Section. (B) Plot for the changes in the intensity of oxidation current peaks over time. (C) Plot for the changes in fluorescence intensity at an emission wavelength of 432 nm over time. Error bars indicate the standard deviation of triplicate measurements ($n = 3$).

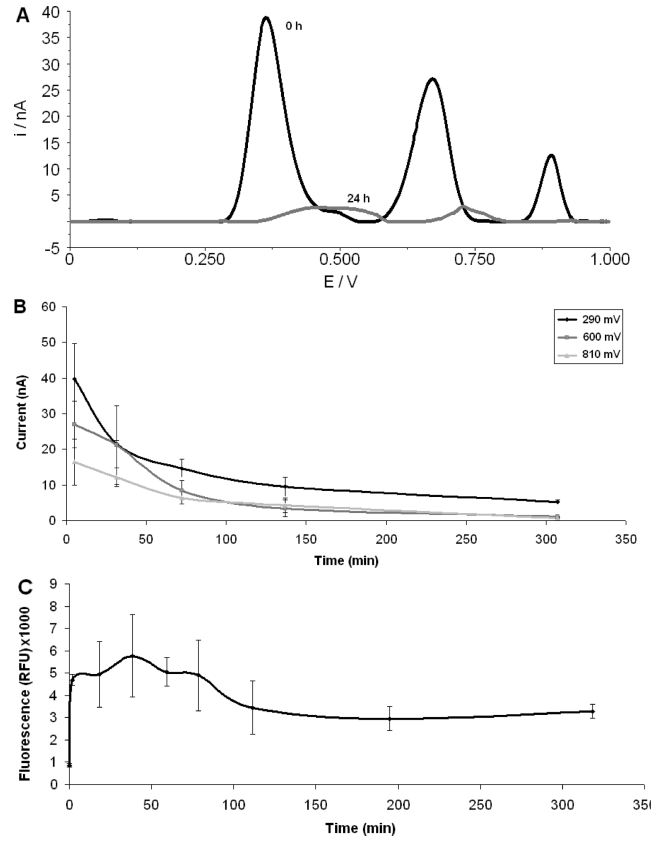

Figure 3. (A) DPV voltammograms of 50 μM BTA-1 incubation in the presence of 50 μM Aβ-42 at 37 °C in 50 mM PBS (pH 7.40) measured using SPCSs at predetermined time intervals. (B) Plot for the changes in the intensity of oxidation current peaks over time. (C) Plot for the changes in fluorescence intensity at an emission wavelength of 432 nm over time. Error bars indicate the standard deviation of triplicate measurements ($n = 3$).

in the ThT and BTA-1 oxidation signals in the absence of the Aβ peptides under similar incubation conditions. The detailed results are presented in the Supporting Information. ThT control samples did not show any change in the anodic peak current intensities over 24 h of incubation. BTA-1 control sample (Figure 5B in the Supporting Information) displayed a negligible decay in anodic peak current intensities over time; however, the decrease of BTA-1 oxidations signals observed in the presence of the Aβ peptides occurred at a significantly faster rate. For the confirmation of electrochemical analysis, fluorescence measurements were performed (Figure 2C) and the results indicated an immediate increase in fluorescence upon incubation of BTA-1 with Aβ-40, in agreement with the literature results.[7] Fluorescence intensity of the BTA-1 with Aβ-40 sample reached a maximum value of 65 000 RFU after 2 h of incubation.

The results of incubation of BTA-1 with Aβ-42 are given by Figure 3A−C. The addition of Aβ-42 to BTA-1 (Figure 3A) did not result in an immediate decrease in peak currents, as it did with Aβ-40 incubation. The peak currents of all three BTA-1 peaks were approximately equal to those observed in the control. The oxidation signal of BTA-1 in the presence of Aβ-42 (Figure 3B) was also found to decay exponentially but did so at a much greater rate than both the BTA-1 in the presence of Aβ-40 and the BTA-1 alone control sample. This was concurrent with the expected rate of aggregation of Aβ-42 due to its much greater hydrophobic nature relative to Aβ-40.[3] This increased rate of aggregation of Aβ-42 was also observed in fluorescence analysis (Figure 3C). The maximum fluorescence emission of 5000 RFUs was reached almost immediately for BTA-1 with Aβ-42, whereas reaching the maximum fluorescence of BTA-1 with Aβ-40 required 2 h of incubation time. However, the increase in fluorescence signal of BTA-1 was not as high for Aβ-42 aggregation as that observed for Aβ-40.

The electrochemical signals observed during the incubation of ThT with Aβ-40 are shown in Figure 4A−C. The addition of Aβ-40 to ThT (Figure 4A) did not result in an immediate decrease in peak currents as those observed with BTA-1 and the Aβ peptides. Conversely, the oxidation signal of ThT was found to increase from 185 to 200 nA after 5 h of incubation (Figure 4B) rather than the expected decrease that was observed for BTA incubated samples. Fluorescence analysis of ThT during the Aβ-40 aggregation (Figure 4C) indicated that a maximum fluorescence intensity of 190 RFU was reached after 2 h of incubation.

The increase in oxidation signal of ThT was again observed in the incubation of ThT with Aβ-42 (Figure 5A). While the maximum oxidation signal of the ThT with Aβ-40 was reached

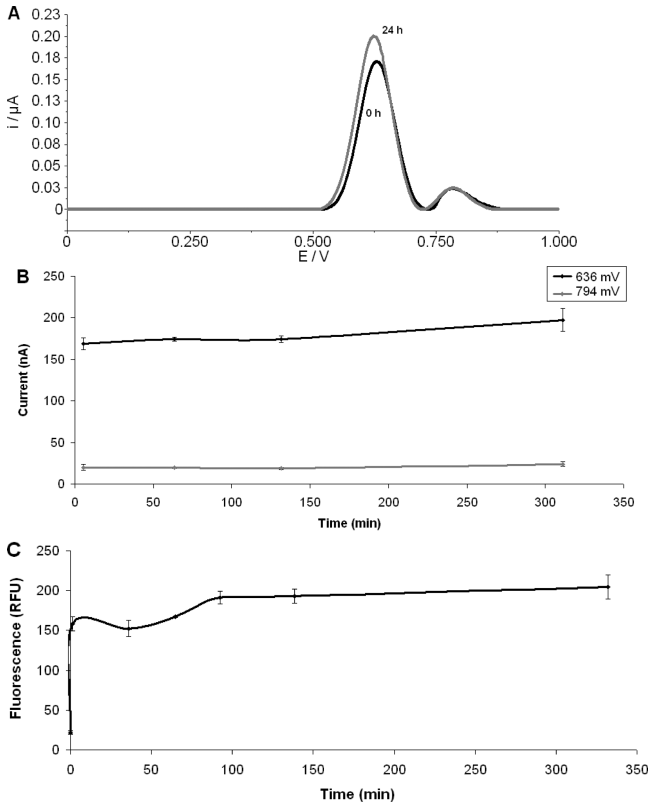

Figure 4. (A) DPV voltammograms of 50 μM ThT incubation in the presence of 50 μM Aβ-40 at 37 °C in 50 mM PBS (pH 7.40) measured using SPCSs at predetermined time intervals. (B) Plot for the changes in the intensity of oxidation peaks over time. (C) Plot for the changes in fluorescence intensity at an emission wavelength of 490 nm over time. Error bars indicate the standard deviation of triplicate measurements ($n = 3$).

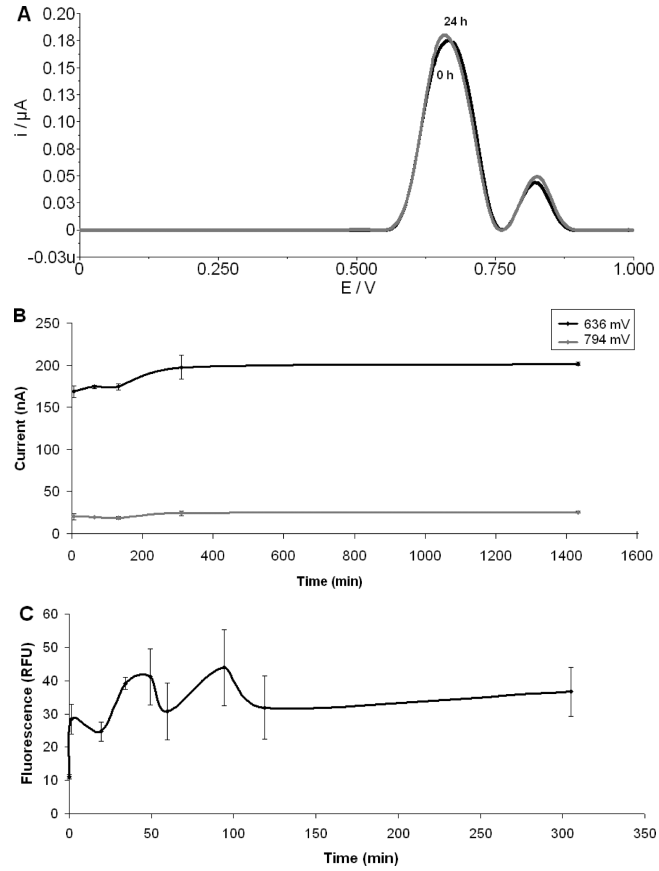

Figure 5. (A) DPV voltammograms of 50 μM ThT incubation in the presence of 50 μM Aβ-42 at 37 °C in 50 mM PBS (pH 7.40) measured using SPCSs at predetermined time intervals. (B) Plot for the changes in the intensity of oxidation peaks over time. (C) Plot for the changes in fluorescence intensity at an emission wavelength of 490 nm over time. Error bars indicate the standard deviation of triplicate measurements ($n = 3$).

after 5 h of incubation, the maximum signal of ThT with Aβ-42 was reached after only 2 h of incubation. This was once again indicative of the more rapid aggregative properties of Aβ-42 in comparison to Aβ-40. This different aggregation characteristic was further supported with the fluorescence analysis (Figure 5C), in which the maximum fluorescence intensity of ThT with Aβ-42 was reached immediately upon incubation, while the maximum fluorescence of ThT with Aβ-40 occurred after 2 h of incubation.

The increase in the oxidation signal of ThT was unexpected; however, reports on the binding sites of ThT and BTA-1 to amyloid peptides suggests possible reasons for its occurrence.[7,18] Wu et al.[7] identified three sites of binding for ThT and BTA-1 to amyloid fibrils: (1) along the side of β-sheets, (2) along the center of the fibril strand, and (3) at the ends β-sheets. Binding toward the center and sides of the fibrils has been found to be the predominant mode of binding. Despite the hydrophobicity of the β-sheet pockets, the polar ThT molecule is able to bind, though to a lesser extent than the hydrophobic BTA-1. We hypothesized that ThT initially intercalated with poor affinity to the center, side, and ends of the β-sheets of individual peptides at the early stages of the aggregation. However, as the aggregation progresses and fibrils begin to align against one another, ThT is less likely to remain at the center of the hydrophobic fibrils, as the initial binding affinity is already very low. The ThT moves out of the center of the sheets and is forced to occupy the smaller area of the outer surfaces of the fibrils. However, since the concentration of ThT in solution remains approximately constant during the incubation process, this increases the ThT accumulation on the fibrils, where they became more available to electrode surface, thereby increasing the oxidation signal of ThT, as aliquots (20 μL) of the peptide/dye mixture were taken out of the solution and measured at a new electrode surface over the incubation period. This hypothesis would also explain the decrease in the BTA-1 oxidation signal. The hydrophobic BTA-1 has the tendency to intercalate and remain at the center of the hydrophobic core of the fibrils.[7] This behavior reduced the availability of BTA-1 to the electrode surface, thereby reducing the oxidation signal over time.

CONCLUSIONS

Amyloid formation is a prominent histopathological development of neurodegenerative diseases such as AD at the early stages. In an effort to develop an electrochemical method of detection, we performed oxidation measurements of well-described intercalative dyes ThT and BTA-1 in the presence and absence of amyloid peptides. Dramatically different behaviors of the positively charged ThT and its neutral analogue BTA-1 were observed on carbon surfaces. These observations provide a new electrochemical point of view to the well-known optical characteristics of these compounds. Our results also demonstrate the utility of electrochemical analysis

as an effective tool for the discovery and screening of novel compounds that can be synthesized to target the amyloids in vitro. Research efforts toward the synthesis and screening of new intercalative dyes are in progress in our laboratory.

ACKNOWLEDGMENT

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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