Journal of Chromatography B, 879 (2011) 627–632

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Analysis of Aβ (1-40) and Aβ (1-42) monomer and fibrils by capillary electrophoresis

Ryan A. Picou[a], Indu Kheterpal[b], Amber D. Wellman[c], Madhavi Minnamreddy[b], Ginger Ku[b], S. Douglass Gilman[a,*]

[a] Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803, USA
[b] Protein Structural Biology, Pennington Biomedical Research Center, LSU System, Baton Rouge, LA 70808, USA
[c] Department of Chemistry, University of Tennessee, Knoxville, TN 37996-1600, USA

ARTICLE INFO

Article history:
Received 30 September 2010
Accepted 22 January 2011
Available online 2 February 2011

Keywords:
Capillary electrophoresis
UV absorbance
Amyloid beta peptide
Protein aggregation
Mass spectrometry

ABSTRACT

A method based on capillary electrophoresis (CE) with UV absorbance detection is presented to characterize synthetic amyloid beta (Aβ) peptide preparations at different aggregation states. Aggregation of Aβ (1-40) and Aβ (1-42) is closely linked to Alzheimer's disease (AD), and studying how Aβ peptides self-assemble to form aggregates is the focus of intense research. Developing methods capable of identifying, characterizing and quantifying a wide range of Aβ species from monomers to fully formed fibrils is critical for AD research and is a major analytical challenge. Monomer and fibril samples of Aβ (1-40) and Aβ (1-42) were prepared and characterized for this study. The monomer-equivalent concentration for each sample was determined by HPLC-UV, and aggregate formation was confirmed and characterized by transmission electron microscopy. The same samples were studied using CE with UV absorbance detection. Analysis by mass spectrometry of collected CE fractions was used to confirm the presence of Aβ for some CE–UV peaks. The CE–UV method reported here clearly indicates that monomeric and aggregated Aβ were electrophoretically separated, and substantial differences in the electrophoretic profiles between samples of Aβ (1-40) and Aβ (1-42) were observed. This CE–UV method can differentiate between Aβ monomer, oligomeric intermediates, and mature fibrils.

1. Introduction

Amyloid beta (Aβ) peptides have been identified as the primary peptide component of the neuritic plaques of Alzheimer's disease (AD) patients [1–3]. This has led to the hypothesis that Aβ peptide aggregates are the cause of the development and progression of AD. Biologically, monomeric Aβ is formed through the enzymatic cleavage of the transmembrane amyloid precursor protein (APP) [4]. Different length Aβ peptide monomers can be formed from APP through additional enzyme processing [4]. The characteristic plaques of AD patients are composed of both Aβ (1-40) and Aβ (1-42), but Aβ (1-42) is the dominant species [5]. Under physiological conditions, soluble Aβ monomers that have been released into the extracellular fluid can self-assemble to form Aβ aggregates, reaching 10–12 nm in diameter and 10^2–10^3 μm in length for mature Aβ fibrils. The toxicity of Aβ is thought to be related to aggregate size and structure, and the most toxic species may be an intermediate species between monomer and fibrils [3,6–8].

Both Aβ (1-40) and Aβ (1-42) peptides have been the focus of intense research because of their relevance to AD and their potential as therapeutic targets for AD treatment. The characterization of Aβ aggregation is complex and challenging, even when synthetic peptides of only one length (e.g. Aβ (1-40) or Aβ (1-42)) are studied. Preparations of aggregated, synthetic Aβ peptides result in heterogeneous mixtures containing numerous aggregated structures and unaggregated monomer. In order to understand the aggregation process and determine which Aβ aggregate species are toxic, it is necessary to characterize and quantify the different aggregate species present in a sample. Few analytical techniques are capable of analyzing Aβ monomer and aggregates with a single experiment. Thioflavin T (ThT) fluorescence, transmission electron microscopy (TEM) and light scattering, for example, are powerful tools for analyzing large Aβ aggregates but are not well suited for studying small oligomeric structures and monomer [9]. Separation techniques such as HPLC, size-exclusion chromatography and gel electrophoresis have been applied to Aβ analysis [5,9]; however, these methods are able to separate Aβ structures only over a limited size range. Their stationary phases have the potential to disrupt aggregates during the separation [10]. An ideal separation method for Aβ analysis would provide rapid and gentle separations of structures ranging in size from monomeric Aβ to mature Aβ fibrils.

Capillary electrophoresis (CE) has been used to analyze species ranging in size from small cations like Na^+ and K^+ to whole cells

* Corresponding author. Tel.: +1 225 578 3010; fax: +1 225 578 3458.
E-mail address: sdgilman@lsu.edu (S.D. Gilman).

1570-0232/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2011.01.030

[11–13]. Capillary electrophoresis with UV absorbance detection (CE–UV) is emerging as a valuable tool for studying Aβ peptides [14–19]. In 1993, Sweeney et al. first applied CE–UV to analyze Aβ peptides [14]. Over a decade later, Verpillot et al. produced similar CE–UV results and additionally analyzed Aβ (1-40) peptide from cerebrospinal fluid of an AD patient [15]. De Lorenzi and coworkers analyzed Aβ (1-40) and Aβ (1-42) peptides by CE–UV after performing ultracentrifugation using different molecular weight cut-off filters to determine the sizes of aggregates producing peaks attributed to Aβ aggregates [16]. The antifibrillogenic effectiveness of small molecules on Aβ (1-42) peptides by CE–UV was also studied using a similar approach [17]. Picou et al. recently reported a CE–UV method to characterize and quantify Aβ (1-40) monomer samples and predict whether an Aβ preparation will exhibit normal or accelerated aggregation kinetics [19]. While previous papers have shown that CE–UV has the potential to serve as a powerful tool for studying Aβ aggregation, such studies are challenging. Small changes in sample preparation can result in large variations in aggregation kinetics and aggregate structures [5,19,20]. In this paper, stringent preparation procedures were used to prepare high quality Aβ monomer and aggregated samples at low concentrations (≤25 μM). The Aβ concentrations were determined independently by HPLC with UV absorbance detection (calibration based on amino acid analysis (AAA)), and the aggregated samples were characterized by TEM. This study assesses the potential of CE–UV to distinguish the aggregate types present in different Aβ samples.

2. Materials and methods

2.1. Chemicals

All solutions were prepared in 18 MΩ water obtained from a Modulab water purification system (United States Filter Corp.; Palm Desert, CA) unless otherwise noted. Tris(hydroxymethyl)aminomethane (Tris) and methanol (99.8%) were purchased from Fisher Scientific (Fair Lawn, NJ). Tris buffer was prepared at 10.00 mM, and the pH was adjusted to 7.79 with 1.0 M HCl and filtered through a 0.2 μm filter (Whatman; Hillsboro, OR). This Tris buffer was used for all experiments as the electrophoresis buffer unless otherwise noted. Mesityl oxide (MO) was purchased from Alfa Aesar (Ward Hill, MA), and solutions of MO were prepared in Tris buffer at a concentration of 0.2% (v/v) MO. Formic acid was obtained from Acros Organics (Geel, Belgium). α-cyano-4-hydroxycinnamic acid (CHCA) was prepared in acetonitrile/H_2O (50:50, v/v) containing 1.0% trifluoroacetic acid (TFA) with a final concentration of 10 mg/mL.

2.2. Aβ peptide sample preparations

Aβ (1-40) was purchased from the W.M Keck Foundation Biotechnology Research Laboratory (Yale University; New Haven CT), and Aβ (1-42) peptides were purchased from rPeptide (Bogart, GA). The Aβ (1-42) sequence is shown below, and the Aβ (1-40) sequence is identical except the two amino acids at the C terminus, isoleucine (I) and alanine (A), are not present.

Aβ (1-42)

1 5 10 15 20
D A E F R H D S G Y E V H H Q K L V F F A
25 30 35 40
E D V G S N K G A I I G L M V G G V V I A

Five sample types were prepared for this work: Aβ (1-40) monomer, Aβ (1-40) mature fibrils, Aβ (1-40) seed prepared by ultrasonicating mature fibrils, Aβ (1-42) monomer and Aβ (1-42) mature fibrils. The samples were prepared as described previously by O'Nuallian et al. and Picou et al. [9,19]. Briefly, Aβ peptides were treated with TFA/hexafluoroisopropanol (HFIP) to remove any preexisting aggregates. For Aβ monomer samples, the solvent was evaporated off, and the peptides were dissolved in 10.00 mM Tris at pH 7.79. For Aβ (1-40) aggregate samples, the TFA/HFIP was evaporated off, and the peptides were dissolved stepwise in equal volumes of 2.0 mM NaOH and 2 × phosphate buffered saline (PBS) containing 22.8 mM phosphate, 274 mM NaCl, 5.4 mM KCl and 0.1% NaN_3 at pH 7.4. The samples were centrifuged at 50,000 × *g* for a minimum 10 h at 4 °C. The supernatant was incubated at 37 °C for 7 d. Fibril formation was monitored using HPLC-UV and ThT fluorescence as described previously [9]. The seed sample was prepared by ultrasonicating a mature fibril sample for 30 s with a Branson Digital Sonifier Microtip (Model 450) and then placed on ice for 1 min. The ultrasonication process was repeated 5×. Prior to CE analysis, the fibril and seed samples were buffer exchanged from PBS to electrophoresis buffer, 10.00 mM Tris at pH 7.79 as described previously [21].

The Aβ (1-42) monomer and fibril samples were prepared using the same procedure with the following exceptions: (1) before aggregation, the Aβ (1-42) fibril sample was centrifuged for 30 min at 20,000 × *g* and 4 °C instead of 10 h at 50,000 × *g* and 4 °C, and (2) the Aβ (1-42) fibril sample was incubated for 2 days to form mature fibrils. These method changes were due to the faster aggregation kinetics for Aβ (1-42) peptide compared to Aβ (1-40) peptide.

Table 1
Monomer-equivalent concentrations determined by HPLC-UV.

Peptide type	Sample ID	Monomer equivalent concentrations by HPLC-UV (μM)
Aβ (1-40)	Monomer	20
	Fibril	25
	Seed	20
Aβ (1-42)	Monomer	22
	Fibril	22

2.3. Characterization and quantification of Aβ samples

The Aβ concentration for each sample was determined with a Shimadzu HPLC-UV instrument with detection at 215 nm. The concentration of Aβ standards for calibration was determined independently by AAA, as described previously [22]. For aggregate-free samples, the monomer concentration was determined using peak areas and the standard curve for Aβ. For aggregate-containing samples, the Aβ monomer-equivalent concentration was determined by (1) disassembling the aggregates to form monomer by treatment with 70% formic acid, and (2) determining the resulting monomer concentration by HPLC-UV. The monomer-equivalent concentrations of the samples used in this work are reported in Table 1. Mature fibrils and seed were characterized by TEM and ThT fluorescence assays [23].

2.4. Aβ Analysis by MALDI-MS

Mass spectrometry experiments were performed on an Applied Biosystems Voyager DE-PRO™ MALDI-TOF MS, equipped with a 20 Hz repetition rate nitrogen laser (337 nm). The instrument was controlled by Voyager Version 5.0 Software with Data Explorer™ and was operated in linear mode. The CHCA matrix was prepared as described in Section 2.1. The CE separation buffer for these experiments was 10 mM Tris–HCl at pH 8.0, and a Beckman Coulter CE-MDQ was used for CE experiments. Fractions were collected from 30 consecutive CE separations and combined with an equal volume of matrix. This sample was spotted on a 100 well stain-

less steel MALDI sample plate for MS analysis. The laser power was adjusted for each sample spot with 200 shots acquired per spectrum.

2.5. Aβ Analysis by CE–UV

Capillary electrophoresis with UV absorbance detection was performed with a Beckman Coulter P/ACE MDQ CE system equipped with a diode array detector (DAD) (Brea, CA) [19]. All electropherograms were plotted at 190 nm. The instrument and data collection were controlled with Beckman Coulter 32 Karat™ Software Version 5.0. Fused-silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ). The capillary (ID = 50 μm, OD = 366 μm) was cut to 63.0 cm total length with a window created 53.0 cm from the inlet end using a window maker (MicroSolv Technology Corp.; Eatontown, NJ). The capillary was conditioned by flushing with 1.0 M NaOH (20.0 psi for 1.0 h), 18 MΩ water (20.0 psi for 1.0 h) and Tris buffer (20.0 psi for 30 min).

All samples were dissolved or buffer exchanged (see Section 2.2) into electrophoresis buffer prior to CE in order to eliminate conductivity and composition differences between the sample buffer and electrophoresis buffer. The Tris electrophoresis buffer has a low ionic strength compared to PBS, and the high conductivity of PBS results in a high electrophoretic current and poor results due to excessive Joule heating. Samples (50 μL) were placed in 200 μL thermowell polypropylene vials (Corning Incorporated; Corning, NY). Prior to each run, the sample was removed from the MDQ and vortexed briefly to resuspend any aggregates that settled to the bottom of the sample vial. For each run, MO was injected for 2.0 s at 0.3 psi prior to the Aβ sample injection. The Aβ sample was then injected for 5.0 s at 0.5 psi. The calculated Aβ injection volume was 4.2 nL. Because MO migrated faster than all forms of Aβ, injection of MO first minimized potential on-column interaction between MO and Aβ. The capillary was thermostatted at 20 °C. The applied separation voltage was 25.0 kV (397 V/cm), and the current was 5.0 μA. The detection scan rate was 32 Hz (maximum allowed), and the run time was 10 min.

All electropherograms were plotted as a function of electrophoretic mobility, μ_{ep}, instead of migration time to correct for any electroosmotic flow (EOF) variations, which is a common problem for CE [19,24].

3. Results and discussion

The goal of the studies presented here was to determine if CE–UV could be used to separate and characterize monomeric and aggregated samples of Aβ (1-40) and Aβ (1-42) peptides for carefully prepared and well-characterized samples. In this study, samples of Aβ (1-40) monomer, mature fibrils and seed (ultrasonicated fibrils) and Aβ (1-42) monomer and fibrils were analyzed using CE–UV. The equivalent monomer concentrations of all samples, as measured by HPLC-UV, were kept at low concentrations between 20 and 25 μM Aβ, to minimize structural differences resulting from Aβ concentration differences [25]. Each sample was quantified independently by HPLC-UV and characterized by TEM. Mass spectrometry was used to confirm that selected peaks in the electropherograms were due to Aβ peptide.

3.1. Aβ (1-40) monomer, fibril and seed analysis by CE–UV

A representative electropherogram with UV absorbance detection (plotted at 190 nm) of an Aβ (1-40) monomer sample is presented in Fig. 1a. Monomeric Aβ (1-40) was dissolved directly in electrophoresis buffer (10.00 mM Tris at pH 7.79) and analyzed by CE–UV. The CE conditions are summarized in Table 2. All electropherograms are plotted as absorbance vs. electrophoretic mobility,

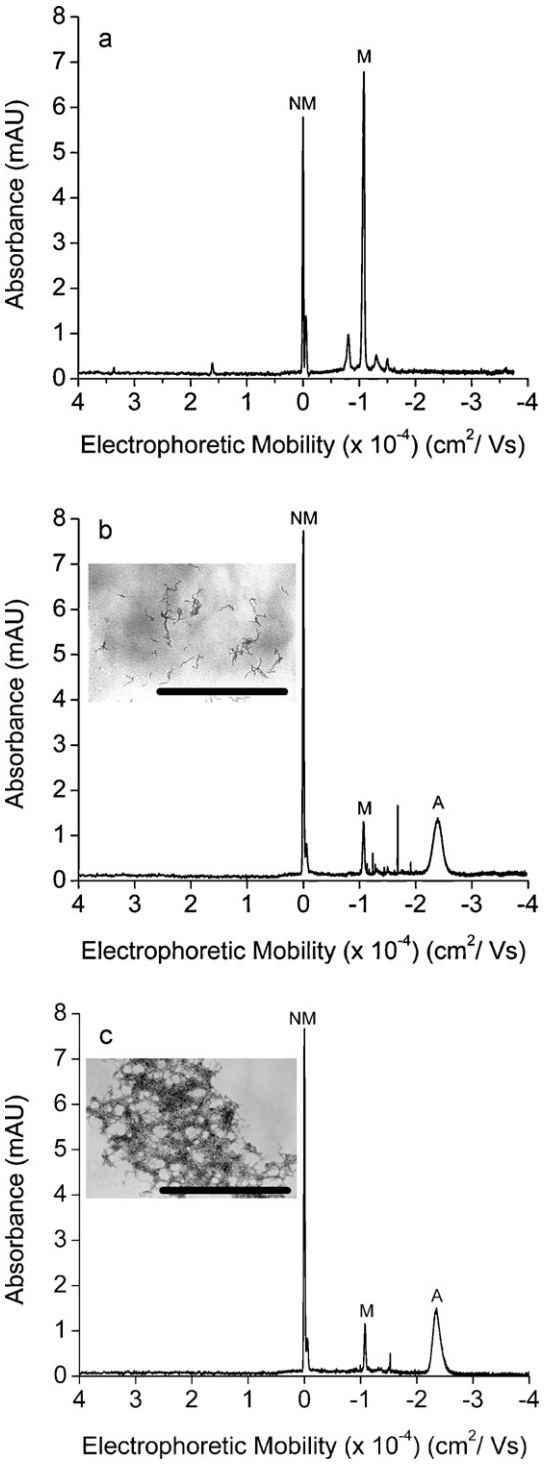

Fig. 1. . Electropherograms of Aβ (1-40) samples. Absorbance was plotted at 190 nm. (a) Aβ (1-40) monomer, (b) Aβ (1-40) fibril, and (c) Aβ (1-40) seed. The neutral marker, NM, has an electrophoretic mobility of 0 $cm^2/V s$. A monomer peak, M, was detected in all Aβ (1-40) samples at $\mu_{ep} = -1.077\ (\pm 0.006) \times 10^{-4}\ cm^2/V s$ ($n = 9$). An aggregate peak, A, was detected in the fibril and seed samples at $\mu_{ep} = -2.37\ (\pm 0.02) \times 10^{-4}\ cm^2/V s$ ($n = 6$). The TEM insets confirm the presence of aggregates observed in the electropherograms (scale bar = 1 μm).

where the neutral marker (NM) has $\mu_{ep} = 0\ cm^2/V s$. The principal peak at a mobility of $-1.082 \times 10^{-4}\ cm^2/V s$ in Fig. 1a is Aβ (1-40) monomer (M). The electrophoretic mobility of the main monomer peak is consistent with that from our previous studies ($-1.2 \times 10^{-4}\ cm^2/V s$) [19]. Fig. 1a does not indicate the presence

Table 2
Summary of capillary electrophoresis conditions.

Capillary dimensions (L_T, L_D, ID)	63.0 cm, 53.0 cm, 50 μm
Separation buffer (Tris concentration, pH)	10.00 mM, 7.79
Aβ injections (t_{inj}, pressure)	5 s, 0.5 psi
Applied electric field	397 V/cm
Electrophoretic current	5.0 μA
Electroosmotic flow velocity ($n = 5$)[a]	0.25 ± 0.01 cm/s
Capillary temperature (thermostatted)	20 °C

[a] The EOF value is an average from the 5 electropherograms shown in Figs. 1 and 3.

of aggregates, which is expected for this sample. Furthermore, the electrophoretic peak pattern of the Aβ (1-40) monomer sample is similar to that observed by Sabella et al. for Aβ (1-40) at a higher Aβ concentration (100 μM) dissolved in 20 mM phosphate at pH 7.4 and electrophoretically separated using 80 mM phosphate buffer at pH 7.4 [16]. The small peaks near the principal monomer peak are thought to be minor impurities from the peptide synthesis [19]; however, other researchers have hypothesized that peaks with similar mobilities could be due to different oligomerization states of Aβ monomers up to 50,000 Da [16]. Attempts clarify the identification of these minor peaks by dilution-based experiments provided inconclusive results (data not shown).

In related experiments, Aβ (1-40) monomer was analyzed by MALDI-TOF MS to confirm the identity of the main monomer peak based on its measured molecular mass. Fig. 2 shows a mass spectrum for the Aβ (1-40) monomer peak. Fractions at the migration time of the monomer peak were collected from 30 CE runs (Fig. 2 inset) and spotted on a MALDI target for MS analysis. The main MS peak corresponds to the molecular ion, $[M+H]^+$, of the Aβ (1-40) monomer (m/z of 4330.5 Da), and this supports the identification of the main peak in Fig. 1a as Aβ (1-40) monomer. The weak MALDI MS signal is not surprising since the sample volume injected for each CE run was only a few nanoliters.

An electropherogram of the Aβ (1-40) fibril sample is presented in Fig. 1b. This sample was prepared to contain mature fibrils by allowing an aliquot of Aβ (1-40) monomer to aggregate for 7 d. Incubation for 7–10 d is common to produce mature fibrils [9,19,26]. Fig. 1b shows two main peaks at -1.071×10^{-4} cm^2/V s and -2.39×10^{-4} cm^2/V s in addition to the neutral marker peak. The resolution for these two peaks labeled A and M is 3.8. The peak at -1.071×10^{-4} cm^2/V s is identified as monomer based on its electrophoretic mobility. Detecting a small monomer peak is

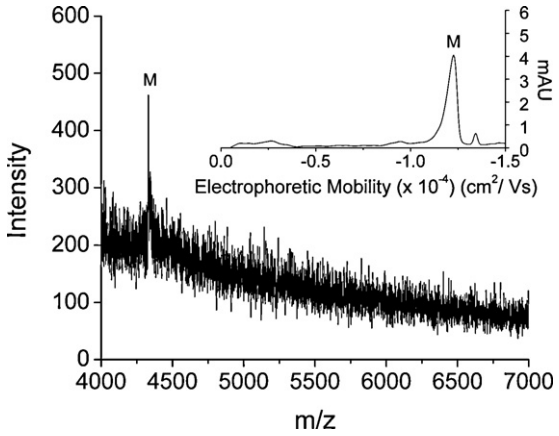

Fig. 2. . MALDI-TOF mass spectrum of collected CE fractions of Aβ (1-40) monomer, M. The Aβ was mixed 1:1 (v/v) with α-cyano-4-cinnamic acid matrix prior to MS. MS data were collected in linear mode. Inset: CE–UV (λ = 200 nm) electropherogram of Aβ (1-40) monomer sample in 10.0 mM Tris–HCl at pH 8.00. The main electrophoretic peak at $\mu_{ep} = -1.2 \times 10^{-4}$ cm^2/V s is monomer, M. Fractions were pooled from 30 consecutive CE runs to obtain the mass spectrum.

not surprising since 0.7–1.0 μM residual monomer remains unaggregated at equilibrium with fibrils [22]. While the monomer equivalent concentrations of the Aβ (1-40) monomer and fibril samples are similar (20 and 25 μM, respectively, Table 1), the monomer peak area in Fig. 1b is reduced relative to that in Fig. 1a, 2.2 mAU s and 15.2 mAU s, respectively. This is expected because Aβ monomer is aggregating to form fibrils, which migrate at different times relative to monomer. The monomer concentration in the Aβ (1-40) fibril sample is estimated to be 3.5 μM based on its peak area (2.2 mAU s). This is four to five-fold greater than the residual monomer concentration reported by O'Nuallian, et al. by HPLC [22].

The broad peak (FWHM = 18.70 s) at -2.39×10^{-4} cm^2/V s in Fig. 1b is attributed to Aβ (1-40) aggregates (A). In previous work, we analyzed several Aβ (1-40) monomer preparations by CE–UV [19]. In that work, some preparations produced only a monomer peak in the CE–UV electropherogram; however, other samples contained an additional broad peak at -2.4×10^{-4} cm^2/V s. These samples also exhibited accelerated aggregation kinetics based on ThT fluorescence studies, suggesting that the additional peak was due to Aβ (1-40) aggregates [19]. Analyses of the Aβ (1-40) aggregate peak by CE–UV and MALDI-TOF MS showed that the CE peak at $\mu_{ep} = -2.4 \times 10^{-4}$ cm^2/V s in mature fibril preparations produced a MS peak at m/z of 4330 Da (data not shown), similar to that shown in Fig. 2. This confirms that the CE peak at -2.4×10^{-4} cm^2/V s contained Aβ (1-40) peptide. The presence of full-length, mature fibrils in the sample studied in Fig. 1b was verified by TEM (inset, Fig. 1b).

An interesting observation in Fig. 1b is the detection of several sharp peaks (FWHM ~0.25 s) with electrophoretic mobilities between those of the monomer and aggregate peaks. These peaks were consistently observed for injections of the Aβ (1-40) fibril sample, but they were not observed for injections of the Aβ (1-40) monomer sample (Fig. 1a). The exact electrophoretic mobilities of these peaks and the number of peaks were quite variable for consecutive injections of the Aβ (1-40) fibril sample compared to the mobility and appearance of the monomer peak and peak A. We hypothesize that these peaks are due to individually detected, large Aβ aggregates. They are similar in appearance to the sharp peaks detected in previous reports for Aβ (1-43) and Aβ (1-42) analyses by CE–UV [14,16,17]. If these sharp peaks are due to individually detected aggregates, then the peak widths will be defined by the migration rate of the aggregate through the detection zone [27]. The optical aperture used in the capillary cartridge defines the detection zone for this work, which is 0.08 cm. The calculated migration rate of the sharp peak in Fig. 1b at -1.7×10^{-4} cm^2/V s is 0.179 cm/s, which was determined by dividing the length of the capillary to the detection window (53.0 cm) by the peak's migration time (295.50 s). The predicted peak width, calculated by dividing the detection zone length by the migration rate of the peak, is 0.5 s. This value is consistent with the measured baseline peak width of 0.45 s. To put this into context, the FWHM and baseline width for the monomer peak in Fig. 1b are 2.25 s and 3.81 s, respectively. For the work in this paper, the instrument's maximum data scan rate was 32 Hz, which means that the 0.45 s peak was represented by about 14 points. Therefore, a scan rate much less than 32 Hz would be insufficient to accurately represent peaks from individual aggregates migrating at the rate described above, based on a minimum of 10 points to define a peak [27,28].

The results in Fig. 1a and b clearly show that the electrophoretic mobility of Aβ peptide changes as it aggregates from monomer to mature fibrils. Both the broad aggregate peak and the sharp peaks have more negative electrophoretic mobilities (i.e. slower migration times) relative to that of the monomer peak. Ideally, the relative sizes of aggregates could be determined by their relative electrophoretic mobilities. Based on the relative migration of the monomer peak and peak A, one might expect that Aβ monomer would be detected first (smaller negative electrophoretic mobil-

ity), followed by the broad aggregate peak labeled A (intermediate negative electrophoretic mobility) and finally the sharp peaks due to mature fibrils (largest negative electrophoretic mobility). However, the sharp peaks migrate between monomer and peak A. For molecules, it is known that electrophoretic mobility is proportional to the ratio of an analyte's charge, z, to hydrodynamic radius, R_h, but it is not known how the ratio z/R_h scales as Aβ aggregates. Additionally, the electrophoretic mobilities of larger Aβ aggregates will depend on more than just their size. Aggregate shape, counter ion double layer and surface zeta potential have been shown to be important for electrophoresis of polystyrene spheres of dimensions similar to mature Aβ fibrils [29]. Because standards for Aβ aggregates do not exist, interpreting separations of Aβ aggregates based on CE and other techniques (e.g. size-exclusion chromatography and field flow fractionation) is quite challenging [30,31].

Seeds are aggregated amyloid peptides that can be added to an unaggregated peptide solution, e.g. a solution of Aβ monomer, to accelerate aggregation to form amyloid fibrils [32]. Seeds reduce the lag phase of Aβ aggregation in a concentration-dependent manner [22,32,33]. An Aβ seed sample was prepared by ultrasonicating mature fibrils as described in the Section 2.2. Ultrasonication breaks apart mature fibrils into smaller pieces. The TEM inset of Fig. 1c confirms the presence of aggregates after ultrasonication. The Aβ (1-40) seed sample was analyzed by CE–UV, and the electropherogram is shown in Fig. 1c. The electrophoretic profile of the Aβ (1-40) seed closely resembles that of Aβ (1-40) fibril (Fig. 1b) with two peaks at -1.072×10^{-4} cm^2/V s and -2.34×10^{-4} cm^2/V s for monomer and aggregates, respectively. The resolution for these two peaks is 4.1. The monomer and aggregate peak areas from the Aβ fibril and seed samples are similar, which is consistent with the samples having similar equivalent monomer concentrations of 25 μM and 20 μM, respectively. For the Aβ (1-40) fibril sample (Fig. 1b), the average monomer and peak A areas are 2.3 ± 0.1 mAU s ($n = 3$) and 20.6 ± 0.5 mAU s ($n = 3$), respectively. The sum of the sharp peak areas in Fig. 1b is 0.6 mAU s for six peaks. For the Aβ (1-40) seed sample (Fig. 1c), the average monomer and peak A areas are 1.7 ± 0.1 mAU s ($n = 3$) and 18.9 ± 0.7 mAU s ($n = 3$), respectively.

Although the electropherograms for Aβ (1-40) fibril and seed are quite similar in most respects, it is interesting to note that the seed sample produced fewer sharp peaks between the mobilities of the monomer and aggregate peaks relative to fibril samples. We hypothesized that the sharp peaks observed in the electropherogram for the Aβ (1-40) fibril sample are due to individually detected mature fibrils. If ultrasonication broke up the larger mature Aβ fibrils producing the sharp peaks, fewer sharp peaks would be observed.

3.2. Aβ (1-42) peptide analysis by CE–UV absorbance

Fig. 3 presents electropherograms for Aβ (1-42) samples. Like Aβ (1-40), Aβ (1-42) is present in neuritic plaques associated with AD, but it is thought to be more neurotoxic compared to Aβ (1-40). It is well known that Aβ (1-42) peptide aggregates much faster than Aβ (1-40) peptide. The Aβ (1-42) monomer sample (Fig. 3a) was prepared as described in Section 2. The sample was analyzed by CE–UV within 6 h of its initial preparation, and during that time, the sample was on ice or refrigerated at 4 °C to minimize aggregation before analysis. A large Aβ (1-42) monomer peak at -1.072×10^{-4} cm^2/V s and a small aggregate peak at -2.29×10^{-4} cm^2/V s were observed in the electropherogram (Fig. 3a). The resolution for these two peaks is 4.1. The TEM in Fig. 3a (inset) confirmed that aggregates were present in the Aβ (1-42) monomer sample despite careful sample preparation and low peptide concentration (22 μM).

Other reports of Aβ (1-42) monomer analysis using CE–UV have shown the presence of aggregate peaks but not a monomer peak in

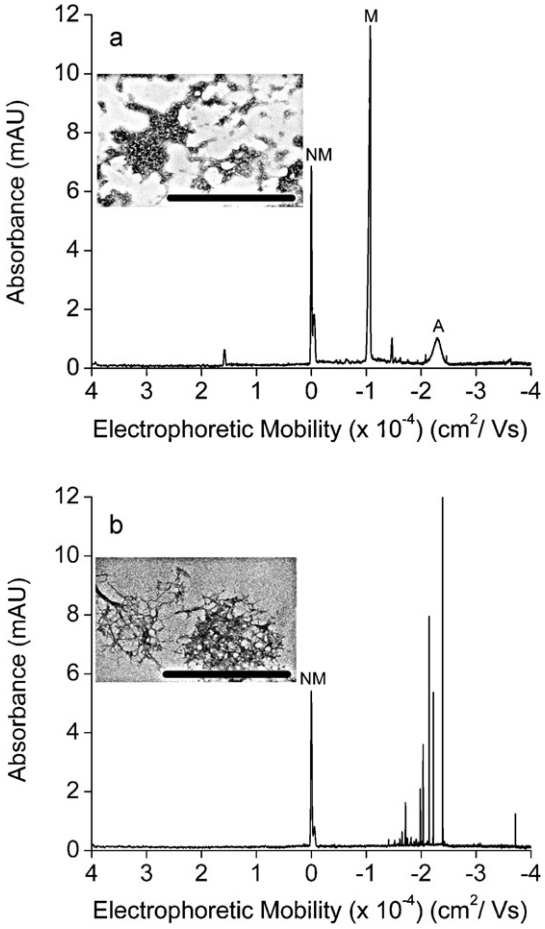

Fig. 3. . Electropherograms of Aβ (1-42) samples. Absorbance was plotted at 190 nm. (a) Aβ (1-42) monomer sample produced a monomer peak, M at $\mu_{ep} = -1.074$ (± 0.003) $\times 10^{-4}$ cm^2/V s ($n = 3$) and an aggregate peak at $\mu_{ep} = -2.294$ (± 0.002) $\times 10^{-4}$ cm^2/V s ($n = 3$). (b) Aβ (1-42) fibril sample produced many peaks in the mobility range of aggregates. The neutral marker, NM, has an electrophoretic mobility of 0 cm^2/V s. The TEM insets confirm the presence of aggregates observed in the electropherograms (scale bar = 1 μm).

contrast to the data shown in Fig. 3a [16,17]. Several factors may explain these differences: sample concentration, peptide source, sample preparation and handling and buffers. De Lorenzi and co-workers used 100 μM Aβ (1-42), about 5 times the concentration used in this work. Their sample was prepared in 20 mM phosphate buffer at pH 7.4, and 80 mM phosphate buffer at pH 7.4 was used as the electrophoresis buffer. In the work presented here, the sample and electrophoresis buffers were 10.00 mM tris at pH 7.79. Consistent with the electropherogram for Aβ (1-42) monomer in Fig. 3a, De Lorenzi and co-workers did report a broad peak at a more negative electrophoretic mobility (longer migration time) compared to the peaks they attributed to small (<50,000 Da) Aβ oligomers. The electropherogram in Fig. 3a indicates that it is possible to prepare and analyze a sample of Aβ (1-42) that contains primarily monomer as indicated by the peak at -1.072×10^{-4} cm^2/V s, in contrast to the work of De Lorenzi et al.

The electrophoretic mobility of the Aβ (1-42) monomer peak in Fig. 3a is almost identical to that for the Aβ (1-40) sample presented in Fig. 1, suggesting that the two additional amino acids in the Aβ (1-42) peptide did not result in a significant mobility shift. The CE method described in this work was not optimized to separate Aβ (1-40) monomer from Aβ (1-42) monomer. Rather, it was designed to separate monomeric Aβ from aggregated Aβ, and it is successful for both Aβ (1-40) and Aβ (1-42) peptides. Other reports

have demonstrated that CE is capable of separating a mixture of different length Aβ peptides, including Aβ (1-40) and Aβ (1-42) [14,15].

Fig. 3b shows an electropherogram for an Aβ (1-42) mature fibril sample. To produce fibrillar Aβ (1-42), this sample was incubated at 37 °C for 2 d in PBS. After incubation, the sample was buffer exchanged to 10.00 mM tris at pH 7.79 as described in Section 2.2. Unlike all of the other electropherograms in this work, there is no monomer peak apparent near -1.1×10^{-4} cm^2/V s. The equilibrium concentration of Aβ (1-42) monomer has been reported as approximately four to five-fold less than that of Aβ (1-40), which may be below the detection limit for Aβ (1-42) monomer using this method [34]. The TEM for the fibril sample (inset in Fig. 3b) indicates the presence of fibrils. Unlike the Aβ (1-40) fibril and seed samples, no broad peak is detected at an electrophoretic mobility near -2.4×10^{-4} cm^2/V s. Instead, there are 17 peaks between -1.4×10^{-4} and -2.9×10^{-4} cm^2/V s (253–314 s). These peaks all have a FWHM of ~0.30 s. Based on these peaks' electrophoretic mobilities and appearance, it is believed that they are due to mature Aβ (1-42) fibrils. Other researchers have reported detecting sharp peaks when analyzing Aβ (1-42) peptide [17]. Colombo et al. analyzed 100 μM Aβ (1-42) peptide in 20 mM phosphate buffer at pH 7.4. In that work, after 24 h of incubation at room temperature, two main oligomer peaks and a few sharp peaks attributed to microprecipitation and fibril deposition were observed [17]. No other reports examining Aβ (1-42) by CE–UV have shown a separation similar to that in Fig. 3b. As discussed in Section 3.1 for Aβ (1-40) fibril samples, the length of the detection zone and data sampling rate are critical when detecting peaks due to individual fibrils passing through the detector.

4. Conclusions

In this paper, five distinct monomeric and aggregated samples of Aβ (1-40) and Aβ (1-42) peptides were analyzed by CE with UV absorbance detection. The results showed that the CE–UV method can separate monomeric and aggregated forms of Aβ based on differences in electrophoretic mobilities. Samples that contained Aβ aggregates as confirmed by TEM, produced a single broad peak, several sharp peaks, or both, in CE–UV separations. The broad peak is attributed to smaller oligomeric Aβ aggregates, and the sharp peaks to larger mature fibrils. Detecting these sharp fibril peaks requires fast data sampling, and faster data collection combined with a narrower detection window should improve the detection of individual fibrils. Capillary electrophoresis with UV detection is a powerful tool to examine the contents of aggregating Aβ samples containing aggregates ranging from monomer to fibrils, and the method and results described here help lay the foundation for future amyloid analysis by CE.

Acknowledgements

This work was supported by Louisiana State University, the National Science Foundation CHE-0505972 (SDG), and the Rosalinde and Arthur Gilbert Foundation/AFAR Award to Indu Kheterpal. Ryan Picou was supported by a Louisiana Board of Regents Fellowship.

References

[1] J. Hardy, D.J. Selkoe, Science 297 (2002) 353.
[2] R.A. Armstrong, P.L. Lantos, N.J. Cairns, Neuropathology 28 (2008) 351.
[3] G.B. Irvine, O.M. El-Agnaf, G.M. Shankar, D.M. Walsh, Mol. Med. 14 (2008) 451.
[4] G. Evin, M.F. Sernee, C.L. Masters, CNS Drugs 20 (2006) 351.
[5] A. Jan, O. Gokce, R. Luthi-Carter, H.A. Lashuel, J. Biol. Chem. 283 (2008) 28176.
[6] D.M. Walsh, D.J. Selkoe, Neuron 44 (2004) 181.
[7] S.S.-S. Wang, A. Becerra-Arteaga, T.A. Good, Biotechnol. Bioeng. 80 (2002) 50.
[8] D.M. Hartley, D.M. Walsh, C.P. Ye, T. Diehl, S. Vasquez, P.M. Vassilev, D.B. Teplow, D.J. Selkoe, J. Neurosci. 19 (1999) 8876.
[9] B. O'Nuallain, A.K. Thakur, A.D. Williams, A.M. Bhattacharyya, S. Chen, G. Thiagarajan, R. Wetzel, Method Enzymol. 413 (2006) 34.
[10] D.K. Clodfelter, M.A. Nussbaum, J. Reilly, J. Pharmaceut. Biomed. 19 (1999) 763.
[11] F. Foret, S. Fanali, A. Nardi, P. Boček, Electrophoresis 11 (1990) 780.
[12] M.A. Rodriguez, D.W. Armstrong, J. Chromatogr. B 800 (2004) 7.
[13] I. Ali, H.Y. Aboule-Enein, V.K. Gupta, Anal. Lett. 39 (2006) 2345.
[14] P.J. Sweeney, J.G. Darker, W.A. Neville, J. Humphries, P. Camilleri, Anal. Biochem. 212 (1993) 179.
[15] R. Verpillot, M. Otto, H. Klafki, M. Taverna, J. Chromatogr. A 1214 (2008) 157.
[16] S. Sabella, M. Quaglia, C. Lanni, M. Racchi, S. Govoni, G. Caccialanza, A. Calligaro, V. Bellotti, E. De Lorenzi, Electrophoresis 25 (2004) 3186.
[17] R. Colombo, A. Carotti, M. Catto, M. Racchi, C. Lanni, L. Verga, G. Caccialanza, E. De Lorenzi, Electrophoresis 30 (2009) 1418.
[18] B.J. Alper, W.K. Schmidt, J. Neurosci. Methods 178 (2009) 40.
[19] R. Picou, J.P. Moses, A.D. Wellman, I. Kheterpal, S.D. Gilman, Analyst (Cambridge, United Kingdom) 135 (2010) 1631.
[20] S.S.-S. Wang, Y.-T. Chen, P.-H. Chen, K.-N. Liu, Biochem. Eng. J. 29 (2006) 129.
[21] I. Kheterpal, S. Zhou, K.D. Cook, R. Wetzel, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 13597.
[22] B. O'Nuallain, S. Shivaprasad, I. Kheterpal, R. Wetzel, Biochemistry 44 (2005) 12709.
[23] H. Naiki, F. Gejyo, Method Enzymol. 309 (1999) 305.
[24] S.D. Gilman, P.J. Chapman, Methods Mol. Biol. (Totowa, N.J.) 339 (2006) 187.
[25] R. Kodali, R. Wetzel, Curr. Opin. Struct. Biol. 17 (2007) 48.
[26] I. Kheterpal, H.A. Lashuel, D.M. Hartley, T. Walz, P.T. Lansbury Jr., R. Wetzel, Biochemistry 42 (2003) 14092.
[27] Y.H. Rezenom, A.D. Wellman, L. Tilstra, C.D. Medley, S.D. Gilman, Analyst (Cambridge, United Kingdom) 132 (2007) 1215.
[28] H. Ahmadzadeh, R. Dua, A.D. Presley, E.A. Arriaga, J. Chromatogr. A 1064 (2005) 107.
[29] S.P. Radko, A. Chrambach, J. Chromatogr. B 722 (1999) 1.
[30] D.C. Rambaldi, A. Zattoni, P. Reschiglian, R. Colombo, E. De Lorenzi, Anal. Bioanal. Chem. 394 (2009) 2145.
[31] D.B. Teplow, Method Enzymol. 413 (2006) 20.
[32] S.J. Wood, W. Chan, R. Wetzel, Biochemistry 35 (1996) 12623.
[33] B. O'Nuallain, A.D. Williams, P. Westermark, R. Wetzel, J. Biol. Chem. 279 (2004) 17490.
[34] J.D. Harper, P.T. Lansbury Jr., Annu. Rev. Biochem. 66 (1997) 385.