Published in final edited form as:

Neuroimage. 2009 October 1; 47(4): 1215–1220. doi:10.1016/j.neuroimage.2009.05.063.

Amyloid Plaques in PSAPP Mice Bind Less Metal than Plaques in Human Alzheimer's Disease

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Abstract

Amyloid beta (A β) is the primary component of Alzheimer's disease (AD) plaques, a key pathological feature of the disease. Metal ions of zinc (Zn), copper (Cu), iron (Fe), and calcium (Ca) are elevated in human amyloid plaques and are thought to be involved in neurodegeneration. Transgenic mouse models of AD also exhibit amyloid plaques, but fail to exhibit the high degree of neurodegeneration observed in humans. In this study, we imaged the Zn, Cu, Fe, and Ca ion distribution in the PSAPP transgenic mouse model representing end-stage AD (N = 6) using synchrotron X-ray fluorescence (XRF) microprobe. In order to account for differences in density in the plaques, the relative protein content was imaged with synchrotron Fourier transform infrared microspectroscopy (FTIRM) on the same samples. FTIRM results revealed a 61% increase in protein content in the plaques compared to the surrounding tissue. After normalizing to protein density, we found that the PSAPP plaques contained only a 29% increase in Zn and there was actually *less* Cu, Fe, and Ca in the plaque compared to the surrounding tissue. Since metal-binding to A β is thought to induce redox chemistry that is toxic to neurons, the reduced metal-binding in PSAPP mice is consistent with the lack of neurodegeneration in these animals. These findings were in stark contrast to the high metal ion content observed in human AD plaques, further implicating the role of metal ions in human AD pathology.

Keywords

Alzheimer's disease; transgenic mice; neurodegeneration; amyloid plaques; metal; zinc; copper; iron; calcium; x-ray fluorescence; infrared microspectroscopy; synchrotron

Introduction

One of the key clinical features of Alzheimer's disease (AD) is the extracellular deposition of amyloid plaques primarily composed of a 39–43 amino acid protein called amyloid beta (A β). A β is derived from the proteolytic cleavage of a larger transmembrane glycoprotein, the amyloid precursor protein (APP), by β and γ secretases (Selkoe 2001). β -secretase first cleaves APP extracellularly, forming the N-terminus, and γ -secretase then cleaves APP in the

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intramembrane domain, creating the C-terminus. The two most frequently produced peptides are A β 40, composed of 40 residues and found mostly in cerebrospinal fluid and vasculature, and A β 42, composed of 42 residues and the main component of amyloid plaques (Roher et al. 1993). The formation of A β , even the deposition of diffuse plaques, is a normal cellular process, but in AD the peptide aggregates to form dense plaques in specific regions of the brain (Masters et al. 2006). But exactly how and why this misfolding occurs is still unknown.

Zinc (Zn), copper (Cu), iron (Fe), and calcium (Ca) ions are all present in the body under normal conditions, but their abundances are elevated in regions of the brain that are involved in AD (Bush 2003; Lovell et al. 1998). The observation that human A β can bind to certain metal ions *in vitro* may indicate these ions play a role in increasing the protein's toxicity and the tendency to form plaques (Bush 2003; Bush et al. 1994; Cuajungco and Faget 2003; Maynard et al. 2005; Miura et al. 2000). Nuclear magnetic resonance (NMR) and X-ray absorption spectroscopy (XAS) studies of metal binding to human A β have revealed the presence of a low and high affinity binding site for both Cu and Zn via three N-terminal histidine imidazole rings and that the stoichiometry for Cu:A β is 1:1 and for Zn:A β ranging from 1:1 to 3:1 (Atwood et al. 2000; Clements et al. 1996; Streltsov 2008).

The affinity for the Zn binding sites were measured as 100 nM and 5 μ M, (Bush et al. 1994) and 10^{-10} M for Cu (Atwood et al. 2000). These affinities are well below physiological levels ($\sim$ 100–150 μ M (White et al. 2006)), supporting the possibility that Zn and Cu are likely to bind human A β *in vivo*, especially in AD where brain metal levels are known to be increased (Suh et al. 2000). Indeed, elevated levels of Zn, Cu, and Fe ions have been observed in amyloid plaques in human AD brain (Lovell et al. 1998; Miller et al. 2006; Suh et al. 2000) and studies involving Swedish mutant APP transgenic mice have shown that Zn is elevated in dense senile plaques (Lee et al. 1999). It has also recently been shown that Zn transporter proteins, which transport Zn ions into different intracellular compartments when intercellular Zn is elevated, are increased in AD and are abundantly expressed in human senile amyloid plaques (Smith et al. 2006; Zhang et al. 2008). Increased Fe was also found within the glial cells surrounding the plaques (Quintana et al. 2006). Excess Fe is typically sequestered by the iron storage protein ferritin and it was found that expression of ferritin is associated with degeneration of microglia in AD brain (Lopes et al. 2008). However, those previous studies primarily utilized histochemical methods, which can only detect free and loosely bound metal ions and cannot be accurately compared to the metal content in the surrounding healthy (non-plaque) tissue. In addition, previous studies that quantified metal content using proton and X-ray methods (Lovell et al. 1998; Miller et al. 2006) did not consider the elevated protein density in the plaque compared to the surrounding tissue. Thus, the observed increase in metal ions within the plaques may simply be attributed to an increase in protein density within the plaque and not an “accumulation” of excess metal ions or metalloproteins.

A number of mouse models have been developed to mimic one or more neuropathological features of AD, which has resulted in valuable progress in understanding AD progression. However, there is some evidence that they are not a complete representation of human AD (Schwab et al. 2004). While mice overexpressing mutant AD related genes exhibit many of the same neuropathological and behavioral features of human AD, including amyloid plaques, neurofibrillary tangles, motor impairments, and memory deficits (German and Eisch 2004; Wirths et al. 2008), most transgenic models lack the widespread neuronal loss and severity of symptoms present in human AD pathology (Takeuchi et al. 2000). Additionally, the surrounding neurons appeared displaced by the plaques rather than damaged by them (Schwab et al. 2004). Exactly why this occurs is not known; however, mouse A β lacks a histidine at position 13. This residue has been shown to be critical in coordinating Cu and inducing aggregation in human A β , where wild-type mice show no aggregation (Atwood et al. 1998; Syme et al. 2004). Transgenic mice develop amyloid plaques that contain both endogenous

murine A β and transgene-derived human A β (Pype et al. 2003; van Groen et al. 2006). Since both types are found in transgenic mouse plaques, this mixture may alter the metal-binding characteristics of the peptide (Radde et al. 2008).

We have previously shown that endstage human AD is characterized by the accumulation of Zn, Cu, Fe, and Ca that are co-localized with areas of high β -sheet protein and that the concentrations of metal inside the plaque are much higher than outside of the plaque (Miller et al. 2006). Here, those results are compared to analogous studies from the end stage of a PSAPP transgenic mouse model of AD, which overexpresses the presenilin (PS) 1 gene and the amyloid precursor protein (APP) gene. Synchrotron X-ray fluorescence (XRF) microprobe was used to image the metal content in the plaques and surrounding non-plaque tissue. In order to normalize to plaque protein density, synchrotron Fourier transform infrared microspectroscopy (FTIRM) was used on the same samples. Results revealed a dramatic decrease in metal binding in the PSAPP plaques compared to the human plaques. When normalized to protein density, human plaques showed elevated Zn, Cu, Fe, and Ca, whereas the PSAPP aggregates contained only elevated Zn. The differences in both neurodegeneration and metal accumulation between human AD and the PSAPP mouse model provide more evidence of the involvement of metal ions in AD.

Materials and Methods

Six female B6C3-Tg(APP_{swe}, PSEN1dE9)85 Dbo/J (PSAPP) mice were obtained from Jackson Laboratory (Bar Harbor, ME). The mice were cared for in accordance with the guidelines set by the BNL Institutional Animal Care and Use Committee and were housed at the Brookhaven Laboratory Animal Facility under standard conditions. At 56 weeks of age the mice were deeply anesthetized with 100 mg/kg 1:10 ketamine:xylazine administered by intraperitoneal (i.p.) injection. Afterwards, they were perfused transcardially with phosphate buffered saline (PBS), resulting in exsanguination and death.

The mouse brain specimens were prepared in a similar manner to the human brain specimens described previously (Miller et al. 2006). The brains were removed, frozen on dry ice, and stored at -80°C until processing. For each sample, 30 μm thick whole brain cryosections were mounted onto Ultralene[®] film (SPEX CertiPrep, Metuchen, NJ) and dried at room temperature. This substrate is free of trace elements that interfere with the XRF microprobe measurements and is also sufficiently IR-transparent. Amyloid plaques were visualized with the fluorescent dye, Thioflavin S. In order to minimize tissue disruption and optimize visualization on Ultralene film, a modified procedure based on the method used by (Gunter et al. 1992) was developed. In short, the tissue sections were first rehydrated with 50% ethanol and allowed to dry completely. A 0.006% solution of Thioflavin S in 50% ethanol was placed on the tissue sample and allowed to stand for two minutes. The sample was then rinsed with 50% ethanol and then carefully rinsed with nanopure water to remove the excess Thioflavin S solution. The sections were dried in air, and the locations of the plaques were visualized by their green fluorescence using epifluorescence microscopy (filter cube excitation: 430 nm, emission: 550 nm). All plaques imaged were 30 – 50 μm in size to ensure that the plaque extended through the whole volume probed by the X-ray beam. Approximately 2–4 plaques per sample were analyzed.

The relative content and distribution of Zn, Cu, Fe, and Ca in PSAPP mouse plaques were imaged with the XRF microprobe at beamline X26A at the National Synchrotron Light Source, Brookhaven National Laboratory (Upton, NY). The synchrotron X-ray beam was tuned to 12 keV using a Si(111) channel-cut monochromator. The monochromatic beam was then collimated to 350 $\mu\text{m} \times 350 \mu\text{m}$ and then focused to approximately 6 $\mu\text{m} \times 10 \mu\text{m}$ using Rhcoated silicon mirrors in a Kirkpatrick-Baez geometry. The sample was placed at a 45° angle

to the incident X-ray beam and X-ray fluorescence was detected with an energy dispersive, 9 element germanium array detector (Canberra, Meriden, CT) oriented at 90° to the incident beam. The sample was approximately 6 cm from the detector. A light microscope objective (Mitutoyo, M Plan Apo 5X) was coupled to a digital CCD camera for sample viewing. Thioflavin S fluorescence was viewed using a commercially available epifluorescence module (Navitar, Rochester, NY). Energy dispersive spectra were collected by raster scanning the sample through the X-ray beam using a dwell time of 30 s/pixel and a step size of 4 μm to provide oversampling. Zn K α , Fe K α , Cu K α , Ca K α fluorescence counts were then extracted from background-corrected energy dispersive spectra. NIST thin-film standard reference materials (SRM) 1832 and 1833 were used for calibration. All data were normalized to variations in incident photon flux by normalizing to changes in I_0 measured by an upstream ion chamber. To confirm that Thioflavin S staining did not cause redistribution or leaching of metal ions in the sample, we scanned several areas of a tissue prior to staining with Thioflavin S. We subsequently stained the tissue and then scanned the sample again in the same area. We did not observe any substantial alterations in the Zn, Fe, or Cu abundance or distribution.

For all plaques that were analyzed with the XRF microprobe, the protein distribution was determined using FTIRM at beamline U10B at the National Synchrotron Light Source, Brookhaven National Laboratory (Upton, NY). A Thermo Nicolet Magna 860 FTIR spectrometer, coupled to a Continuum IR microscope (Thermo Nicolet, Madison, WI), was used with synchrotron light as the infrared source. The microscope was equipped with a matching 32 $\times$ Schwarzschild objective/condenser pair, a motorized x-y mapping stage, an adjustable rectangular aperture, and a mercury cadmium telluride (MCT) detector. The regions to be imaged were the same as those imaged using XRF microprobe and were identified as A β aggregates by Thioflavin S fluorescence. The IR microscope stage was raster scanned through this area with a step size of 4 μm . At each point, an absorbance spectrum was collected in transmission mode in the mid-infrared spectral range (4000–800 cm^{-1}) with a spectral resolution of 8 cm^{-1} and 128 scans co-added. The IR beam size was 10 $\times$ 10 μm . A background spectrum was collected from an area with no substrate or sample in the beam.

The FTIRM data were analyzed using Thermo Nicolet's software Omnic 7.3. To examine the relative protein content in the plaques compared to the non-plaque tissue, each spectrum was integrated from 1490 – 1580 cm^{-1} . This region represents the Amide II absorption band, which arises from the N–H bending and C–N stretching modes of the peptide backbone and is proportional to protein concentration. A linear baseline from 1480 – 1800 cm^{-1} was applied. Both the FTIRM and XRF microprobe maps were then normalized by dividing the entire map by the average background intensity of the non-plaque (healthy) tissue. A Matlab routine was then used to mask out the plaque area based on the Zn K α fluorescence, which corresponded well with the Thioflavin S staining. On average, these areas gave Zn fluorescence intensity about 1.8 times higher than non-plaque tissue. These elevated Zn regions were used to create a mask that would define the plaque area for the Cu K α , Fe K α , and Ca K α images. A similar procedure was followed to create the FTIRM protein images. There were approximately 150 pixels per plaque. Although a faster method would have been to obtain a few individual spectra from the plaque and non-plaque regions, we chose the current procedure because we have previously observed a heterogeneous distribution of metal in plaques. Therefore, a single spectrum or even an average of a few spectra may overestimate or underestimate the amount of metal observed depending on where the spectrum was taken. Once all of the appropriate pixels from each image were masked out, the data were checked for normality with a Kolmogorov-Smirnov test using SPSS v.14.0 and means and standard deviations were calculated. The final data represented a ratio of the intensity in the plaque to the non-plaque area. The percentage increase in metal within the plaque compared to the non-plaque area was also determined. A ratio of metal/protein content for each sample was then calculated. To

compare the actual change in metal content once the plaques were normalized for protein density, the percentage difference was determined.

Results

The distribution of protein, as measured by the Amide II FTIRM band, in three plaques from a PSAPP mouse representing endstage AD is shown in Figure 1. Also shown are representative spectra illustrating the increase in protein in the plaque compared to the surrounding normal tissue. The protein distribution showed that the protein density was highest in the center of the plaque and decreased toward the periphery. On average, the protein density was 61% higher within the PSAPP plaques than the surrounding area (Table 1).

Figure 2 shows the XRF microprobe images of the Zn, Cu, Fe, and Ca distributions from the same plaques as shown in Figure 1. Representative spectra from the plaque and non-plaque regions are also shown. Results show that the relative Zn, Fe, and Ca contents are preferentially concentrated in the core of the plaque, with about 1.8, 1.7, and 1.5 times more, respectively, in the center than the edge of the plaque. In contrast, the relative distribution of Cu was more homogeneous: no central core was observed and, although there were a few elevated spots in some areas, the plaque was generally only about 1.2 times elevated above the background. When compared to the surrounding non-plaque tissue, there was approximately a 107% increase in Zn, 25% increase in Cu, 33% increase in Fe, and a 50% increase in Ca (Table 1). Although Zn, Cu, and Fe have all been implicated in AD plaques, this is the first known study to show elevated Ca in PSAPP plaques. Since A β does not have any known binding sites for Ca, this result implies there are other proteins or physiological processes using Ca in the plaques.

In order to attain the relationship between metal content and protein concentration and to ensure that any changes in protein density within the plaque are taken into account, we normalized the metal content to the amount of protein in the amyloid plaque. Specifically, a ratio of relative metal content to the relative protein content measured by Amide II was calculated for each element and sample (Table 1). Results showed that, when normalized to protein content, there was approximately a 29% increase in Zn compared to protein in the plaque, while there was actually 22% less Cu, 17% less Fe, and 7% less Ca in the plaque.

In order to compare these findings to human AD, the same procedure was applied to the data used in our previous human AD study (Miller et al. 2006). In contrast to PSAPP mouse plaques, human AD plaques are highly enriched in Zn, Cu, Fe, and especially Ca (Table 2). Specifically, when compared to the surrounding tissue, there was approximately a 908% increase in Zn, 1171% increase in Cu, 573% increase in Fe, 9838% increase in Ca. Human plaques showed a 107% increase in protein, so when normalized to protein density, there was still a 339% increase in Zn, a 466% increase in Cu, a 177% increase in Fe, and a 4653% increase in Ca.

Discussion

There is growing evidence to suggest that the accumulation of metals ions in amyloid plaques plays an important role in AD pathology (Lee et al. 1999; Lovell et al. 1998; Miller et al. 2006). However, previous studies used non-quantitative histochemical techniques or did not normalize to increased plaque density, the former of which only identify free metal ions. Our goal in this study was to analyze the distribution of Zn, Cu, Fe, and Ca in PSAPP mouse plaques normalized to the density of the tissue and to compare the results to previous results from our laboratory examining human AD plaques (Miller et al. 2006). Like the human plaques, the present results showed that PSAPP mouse plaques were elevated in Zn, Cu, Fe, and Ca. Interestingly, however, we found that the abundance of metal in the PSAPP plaques was

considerably lower than in the human plaques. Even though human plaques are more dense (i.e. showed approximately 30% more protein) than the PSAPP plaques, it is not enough to account for the large increase in metal content. This suggests that metals may not have the same effect in transgenic mouse models of AD as in human AD.

Double transgenic PSAPP mice express a chimeric mouse/human amyloid precursor protein gene (APP695swe) and a mutant human presenilin 1 (PSEN1) gene allowing them to secrete a human A β peptide, which can be detected by antibodies specific for human sequence within this region (Borchelt et al. 1997). PSAPP mice exhibit markedly accelerated amyloid deposition demonstrating the involvement of presenilin 1 in early onset AD (Borchelt et al. 1997). However, it was recently shown that both human and mouse A β are deposited in the plaques of these mice (van Groen et al. 2006) as well as in the plaques of transgenic mice expressing the London APP mutation (Pype et al. 2003). On the other hand, wild-type mice express only rodent A β and do not develop plaques nor any other AD-like pathology (Johnstone et al. 1991; Syme et al. 2004).

In vitro experiments have shown that A β possesses specific binding sites for Zn, Cu, and to a lesser extent, Fe (Danielsson et al. 2007; Miura et al. 2000; Streltsov 2008; Töugu et al. 2008) although only Zn and Cu co-purify with A β extracted from human AD brain (Opazo et al. 2002). In contrast, rodent A β is not aggregated by Zn, Cu, or Fe (Atwood et al. 1998). Rodent A β is different from human A β in that it contains three amino acid substitutions at positions 5, 10, and 13 (Johnstone et al. 1991), but it appears to be the histidine substitution for arginine at position 13 in the rodent A β that minimizes metal-induced aggregation (Liu et al. 1999). Since PSAPP plaques contain a fraction of rodent A β , the metal coordination within these plaques is likely different from human plaques.

In human A β , high-affinity binding of Cu²⁺ to A β has been shown to slowly modify the peptide and promote precipitation (Atwood et al. 2000). Moreover, while most proteins lose metal ions with decreasing pH, A β accepts Cu under mildly acidic conditions and can displace Zn, which loses affinity at low pH, suggesting a combined role of Cu and acidic environments in the pathophysiology of AD. Alternatively, Cu may bind to A β first and Zn may act as an antioxidant by displacing non-specific Cu²⁺ binding (Cuajungco et al. 2000).

We have previously shown that human AD plaques accumulated high amounts of Cu compared to the surrounding normal tissue (Miller et al. 2006), while the present study showed negligible Cu accumulation in PSAPP mouse plaques. Mildly acidic pH is a common occurrence in the aged human brain and also in response to inflammation (Atwood et al. 1998). Since Cu²⁺ coordination is highly pH dependent (Syme et al. 2004), the low level of Cu binding in the present study suggests that in PSAPP mice either the pH in the brain remains at a physiological level and, therefore, not low enough to allow Cu binding or that Zn binding displaced Cu that was previously bound. Moreover, it appears that Zn may change the conformation of A β so that Cu or Fe cannot access its metal binding sites (Cuajungco and Faget 2003). A β is strongly redox-reactive and generates hydroxyl radicals via Fenton chemistry, and peroxide (H₂O₂) species via the Haber-Weiss reaction, upon the reduction of Cu²⁺ and Fe³⁺, which may cause oxidative stress (Huang et al. 1999). Our human AD study showed nearly equivalent accumulation of Zn, Cu, and Fe, while in PSAPP mice we have shown clearly less Cu and Fe accumulation in the plaques than Zn. This suggests that Cu or Fe binding is important in causing the widespread neuronal damage observed in human AD, which does not occur in PSAPP mice. It is possible that Zn may act as an antioxidant by inhibiting Cu and Fe binding and preventing peroxide formation in these mice.

Although it has been shown that metal binding can form plaques *in vitro*, it is not known if A β *in vivo* is metal bound under normal physiological conditions. However, A β :Cu and

A β :Zn stoichiometry has been shown to be 1:2 and 1:3 respectively *in vitro* with apparent binding affinities in the atto- and picomolar range, indicating that these metals could be bound to A β under normal physiological conditions (Atwood et al. 2000). While we have shown that there appears to be an increase in metal content in plaque versus surrounding tissue, these differences disappear once we consider the amount of protein in the plaque. We have made the assumption that the plaques we analyzed were largely composed of A β based on previous findings using HPLC, laser capture microdissection, and Raman spectroscopy that plaque cores are primarily composed of A β and that other proteins are localized to the periphery or present in minute amounts (Atwood et al. 2002; Dong et al. 2003; Liao et al. 2004; Roher et al. 1993). Thus, since A β is presumed to bind at least 2–3 metal ions per peptide, we expected a minimum of 2–3 times more metal than protein in the plaque. However, this was not the case. We observed an increased accumulation of Zn in the plaque, while Cu, Fe, and Ca were decreased in the plaque when normalized to protein content. One explanation is that once mature plaques have formed, A β may lose affinity for metal ions and this would therefore account for the decrease in metal. It is also possible that the metal binding modes of A β *in vivo* are different from those shown in A β *in vitro*. This is a likely explanation because previous FTIRM studies have shown that amyloid plaques in AD tissue have an elevated β -sheet structure that is different than the A β structure found *in vitro* (Choo et al. 1996; Miller et al. 2006). The different structure of A β in the tissue may contribute to different metal binding modes than those observed *in vitro*. This difference may be even greater in PSAPP mice considering that the amyloid that accumulates in plaques of transgenic AD mice was found to be chemically distinct from that found in human AD (Kalback et al. 2002). In addition, Cu is abundant in human plaques, while lacking in PSAPP plaques. This is an important finding because A β and Cu-bound histidine have been shown to be involved in the generation of neurotoxic H₂O₂ (Dong et al. 2003) and the presence of Cu in the plaques could be one reason why human AD exhibits such severe neurodegeneration, which is not observed in the mice.

Calcium disruption has also been postulated to play a role in AD (Khachaturian 1987) and has gained even more interest lately (Cheung et al. 2008; Green and LaFerla 2008; Kuchibhotla et al. 2008). It is thought that A β is involved in Ca regulation by forming ion channels in the cell membrane allowing Ca to flow into the cell (Arispe et al. 1994). More recently, it was shown that transgenic mice expressing both mutant APP and PSEN1 exhibited calcium overload while mice expressing only one of the transgenes or mice that did not yet have plaques did not (Kuchibhotla et al. 2008). To the best of our knowledge, this is the first study in transgenic AD mice that has examined Ca distribution in plaques. Ca was found to be extremely elevated in human AD plaques and just slightly elevated in PSAPP plaques. Since A β does not have any known binding sites for Ca, this result implies there could be other physiological processes using Ca in the plaques.

In summary, we have found that PSAPP mice accumulate much less metal in their plaques compared to human AD plaques, suggesting that PSAPP mice are affected differently by metals. These results together indicate that PSAPP mice are only a limited representation of human AD plaque pathology. However, the deficits in these mice are extremely beneficial in that they give insights into potential disease mechanisms of human AD. Why AD in PSAPP mice is different than in humans may be due to a number of factors including differences in A β structure and distribution, as well as species differences such as life span, duration of illness, and other pathologic features (e.g. the absence of neurofibrillary tangles in PSAPP mice). Nevertheless, the lack of metal in PSAPP plaques, while abundant in human plaques, indicates that metals may be related to the detrimental neurotoxicity observed in human AD, which is nearly absent in PSAPP mice.

Acknowledgments

The authors would like to thank Ariane Kretlow and Janelle Collins for their skillful technical assistance with the animal dissection and tissue preparation. We are also grateful to Bill Rao at beamline X26A for his support with beamline operation and data collection. This work is funded by the National Institutes of Health Grant R01-GM66873. The National Synchrotron Light Source is funded by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract DE-AC02-98CH10886.

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Figure 1.

(A) Thioflavin S-stained PSAPP mouse brain tissue showing three plaques. (B) Infrared image of the same tissue showing the distribution of protein measured by the Amide II band. (C) Infrared spectra collected from the areas marked with asterisks in (A) and (B), showing the relative amount of protein in the center of a plaque (black) and the surrounding tissue (red). All scale bars are 5 μm .

Figure 2.

(A) Thioflavin S-stained PSAPP mouse brain tissue, also shown in Fig. 1. XRF microprobe images of (B) Zn, (C) Cu, (D) Fe, and (E) Ca distribution in the same tissue. (F) XRF microprobe spectra collected from the areas marked with asterisks in (A) – (E), comparing the center of a plaque (black) to the surrounding tissue (red). All scale bars are 5 μ m.

Table 1
Relative Metal and Protein Content in PSAPP AD Plaques

	Zinc	Copper	Iron	Calcium	Protein
Measured (inside/outside plaque)					
<i>% Difference</i>	2.07 ± 0.34 +107.0	1.25 ± 0.11 +25.0	1.33 ± 0.14 +33.0	1.50 ± 0.23 +50.0	1.61 ± 0.18 +61.0
Normalized					
<i>% Difference</i>	1.29 ± 0.12 +29.0	0.79 ± 0.07 -22.0	0.83 ± 0.09 -17.0	0.94 ± 0.16 -7.0	

Table 2
Relative Metal and Protein Content in Human AD Plaques

	Zinc	Copper	Iron	Calcium	Protein
Measured (inside/outside plaque)					
<i>% Difference</i>	9.09 ± 0.20 +908	11.72 ± 1.24 +1171	5.74 ± 3.31 +573	98.40 ± 31.56 +9839	2.07 ± 0.18 +107
Normalized					
<i>% Difference</i>	4.42 ± 0.38 +339	5.70 ± 0.49 +466	2.79 ± 0.24 +177	47.85 ± 4.10 +4653	