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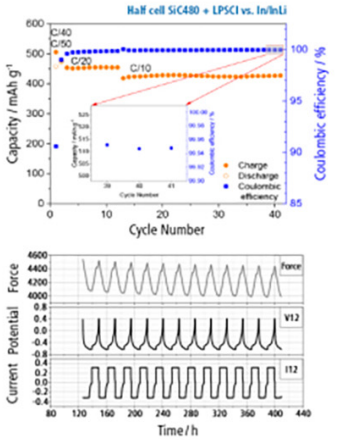

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Amyloid Aβ_{42}, a promoter of magnetite nanoparticle formation in Alzheimer's disease

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Abstract

The accumulation of iron oxides—mainly magnetite—with amyloid peptide is a key process in the development of Alzheimer's disease (AD). However, the mechanism for biogeneration of magnetite inside the brain of someone with AD is still unclear. The iron-storing protein ferritin has been identified as the main magnetite-storing molecule. However, accumulations of magnetite in AD are not correlated with an increase in ferritin, leaving this question unresolved. Here we demonstrate the key role of amyloid peptide Aβ_{42}, one of the main hallmarks of AD, in the generation of magnetite nanoparticles in the absence of ferritin. The capacity of amyloid peptide to bind and concentrate iron hydroxides, the basis for the formation of magnetite, benefits the spontaneous synthesis of these nanoparticles, even under unfavorable conditions for their formation. Using scanning and transmission electron microscopy, electron energy loss spectroscopy and magnetic force microscopy we characterized the capacity of amyloid peptide Aβ_{42} to promote magnetite formation.

Keywords: amyloid peptide Aβ_{42}, magnetite nanoparticle, Alzheimer disease (AD), neurodegenerative brain diseases, metallobiomolecule, iron oxide

(Some figures may appear in colour only in the online journal)

1. Introduction

Alzheimer's disease (AD) is an incurable progressive neurodegenerative disorder characterized by memory loss and impaired cognitive function; it is increasingly being recognized as one of the most important medical problems in the elderly, with a prevalence of AD as high as 25%–50% in those over the age of 85 [1].

During the past decade, many hypotheses have been proposed for the pathogenesis of AD, the β-amyloid (Aβ_{42}) cascade theory being one of the most widely accepted [2]. However, over recent years other possibilities have been studied after a relationship of iron with AD was observed. Iron is the most abundant transition metal in the human brain. We depend on it not only for oxygen transport but also for the underlying formation and maintenance of the neuronal network and for the synthesis of neurotransmitters [3]. While it is essential for maintaining a healthy brain, iron also can have a toxic role. It exacerbates damage to brain tissue following processes such as stroke or trauma. The regional increase of iron within the brains of people with AD, compared with healthy controls, is considered to be a key factor in neuronal atrophy. Regional accumulation and deposition of iron within the brain and altered regulation of iron transporter storage protein are evident in several neurodegenerative diseases, including AD, Parkinson's disease, Huntington's disease and several others [4]. In a recent publication we studied the effect of a complex of magnetite and amyloid Aβ_{42} on the

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spontaneous activity and functional organization of neuronal cultures. Our results showed that a magnetite–Aβ_{42} complex causes a lethal deterioration in the functionality of neuronal circuits [5].

Magnetite, a polycrystalline iron oxide (Fe_3O_4) that contains Fe^{2+} and Fe^{3+} ions, accumulates in Aβ_{42}-rich senile plaques in AD brains. During the complex biochemical cascade that occurs in the development of AD, there is a link between the oxidative stress caused by alterations in the normal metabolism of metals and the precipitation of magnetite in specific sites in the brain [6–8]. Employment of a metal-chelating strategy to interrupt the vicious cycle of production of oxidative stress by metal ions is now considered as a novel alternative therapeutic strategy [9]. Nonetheless, we have no clues about how magnetite is generated in AD-affected brains.

Intense research efforts have been devoted to studying the accumulation of these nanoparticles, and there is an extensive literature on the properties of these metals and their capacity to bind to various proteins [10]. The accumulation of ferrous molecules is associated with the core of the ferritin protein, in which Fe^{2+} is oxidized and stored as ferrihydrite. This protein can store up to 4500 ferrihydrite molecules, which form spherically shaped accumulations, approximately 12 nm in diameter, inside ferritin [11]. This intimate relationship between ferritin and iron leads to the generation of large clusters, which eventually results in the formation of various crystalline ferric species. However, the association between ferritin and the biogeneration of the magnetite present in dementias remains unclear: this is because, the high concentration of magnetite nanoparticles is not correlated with an increase in ferritin concentration in AD brains [12] and the diameter of the magnetite found in AD is larger than that of ferritin core [6]. These observations support the notion that other pathways are involved in magnetite generation.

In previous works we have investigated the interaction between magnetite and Aβ_{42}, two important players in AD. Our results support the hypothesis that the magnetite–Aβ complex is created before the synthesis of the magnetic nanoparticles [8], and this complex is important in the degradation of neurons [5]. Here we studied whether Aβ_{42} peptide promotes the formation of magnetite in the absence of ferritin. The reported ability of Aβ_{42} to attract iron ions in certain regions of the amino acid sequence may help the accumulation of high concentrations of iron, the initiator for magnetite formation, which may tilt the reaction towards magnetite biosynthesis. To probe this hypothesis, we mixed iron ions with Aβ_{42} under an unfavorable pH environment for the spontaneous generation of magnetite nanoparticles. Under these conditions of low pH, far from the more favorable physiological conditions, magnetite synthesis was carried out in the presence and absence of Aβ_{42} in order to study the key role of this peptide. The formation of nanoparticles entrapped in the amyloid fibrils was characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM), electron energy loss spectroscopy (EELS) and magnetic force microscopy (MFM). Magnetite nanoparticle formation only occurred in presence of Aβ_{42}, proving the capacity of amyloid peptide to promote magnetite formation.

2. Experimental section

2.1. Materials

Iron (II) chloride tetrahydrate, iron (III) chloride hexahydrate, ammonium hydroxide, and 1,1,1,3,3,3-hexafluoro-2-propanol were purchased from Sigma-Aldrich. Amyloid β peptide 1–42 (Aβ_{42}) was supplied by EZ-Biolab (Carmel, IN, USA).

2.2. Synthesis of the magnetite–Aβ_{42} complex

Aβ_{42} (0.2 mg) was dissolved in 250 μl of 1,1,1,3,3,3-hexafluoro-2-propanol and divided into aliquots, which were then evaporated with an Eppendorf Concentrator 5301 vacuum centrifuge for 30 min. These conditions promote stability of the Aβ_{42} monomer, as reported by Stine *et al* [13]. Synthesis of the magnetite–Aβ_{42} complex was achieved by mixing 12.5 μmol of $FeCl_3 \cdot 6H_2O$ and 6.25 μmol of $FeCl_2 \cdot 4H_2O$ in 10 μl of double deionized water. This mixture was then added to 0.01 μmol of evaporated Aβ_{42} or to an empty Eppendorf tube for control preparation. We calculated that 1.25 μl of 7.2 M ammonium hydroxide was the volume required to reach a pH of 2 in a 10 μl solution of $FeCl_3/FeCl_2$. Since it was not feasible to check the pH in a volume in the micrometer range, the same pH calculation was performed in a mixture volume that allows pH measurement with a standard pH meter. The solution was mixed at 37 °C for 30 min. Side products from the magnetite–Aβ_{42} complex were removed using consecutive cleaning steps with 1 ml of MilliQ water. Between washing steps, the side products and the unattached amyloid were separated from the magnetite–Aβ_{42} complex by means of a neodymium magnet. The magnetized complex attached to the amyloid was attracted and trapped by the magnet; meanwhile the supernatant with the free peptide and the side products were removed with a micropipette. This step was repeated three times, and the sample was kept in 1 ml of MilliQ water.

2.3. Characterization of the magnetite–Aβ_{42} complex with SEM

SEM images were taken with a Nova NANOSEM 230 FEI microscope (Corvallis, OR, USA) at an accelerating voltage of 10 kV and a working distance of 8 mm.

2.4. Characterization of the magnetite–Aβ_{42} complex with TEM and EELS

20 μl of sample solution was deposited on typical TEM copper grids with holey carbon membrane and allowed to evaporate at room temperature. TEM and EELS images of the magnetite–Aβ_{42} complex were obtained with a JEOL TEM2100 equipped with an Orius SC10001 camera and Oxford Instruments INCAx-Sight detector. A voltage of 200 kV and exposure time of 2 s was used.

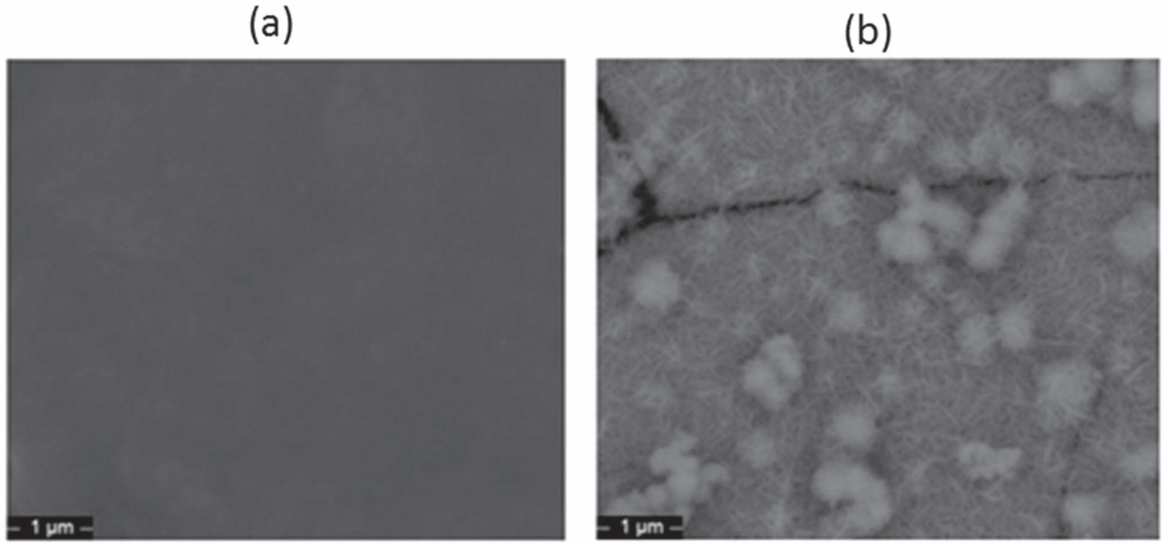

Figure 1. SEM result for magnetite synthesis in the absence (a) and presence (b) of amyloid. Scale bars = 1 μm.

2.5. Characterization of magnetite–Aβ_{42} complex with MFM

MFM images of the magnetite nanoparticles formed in the presence of Aβ_{42} were obtained with a MultiMode Atomic Force Microscope (Bruker) equipped with a 12 μm E scanner. Forty microliters of sample solution was deposited on a cleaved mica substrate and left to adsorb for a few minutes. After being rinsed twice with 40 μl of MilliQ water, the mica substrate was carefully dried under a nitrogen stream. The images were taken in air by scanning the sample with magnetized MESP7 probes (Bruker) in tapping mode at scan rates in the range 0.5–2 Hz. Height, amplitude and phase images were recorded simultaneously. Phase images were obtained by lifting the probe 100 nm above the sample topography during scanning.

3. Results and discussion

We studied the key role played by Aβ_{42} in the formation of magnetite nanoparticles in AD. Magnetite nanoparticles were synthesized by means of the co-precipitation of Fe^{2+} and Fe^{3+} with hydroxide, as the precipitation agent, obtaining the formation of iron hydroxide with the subsequent conversion to magnetite as [14]

$$Fe^{2+} + 2OH^- \rightarrow Fe(OH)_2$$

$$Fe^{3+} + 3OH^- \rightarrow Fe(OH)_3$$

$$Fe(OH)_2 + 2Fe(OH)_3 \rightarrow Fe_3O_4 + 4H_2O.$$

Amyloid peptide Aβ_{42} has been reported to be able to create complexes with iron hydroxide in both species, Fe^{2+} and Fe^{3+}, which helps to stabilize these iron ions [15]. But this is a symbiotic interaction that also contributes to the aggregation and stabilization of amyloid monomers into fibrils [16]. In this work, we examined whether the formation this beneficial interaction promotes the synthesis of magnetite. To probe the importance of amyloid in the formation of magnetite nanoparticles, unfavorable conditions for this synthesis were applied in the presence and absence (control) of Aβ_{42}.

Several variables tilt the direction of this synthesis towards magnetite formation. Thermodynamics is highly relevant in all chemical reactions to achieve efficient reaction kinetics, thus an increase in the temperature contributes to faster formation of the product. The law of mass action also speeds up the chemical reaction when the amount of reacting substances present is increased. Therefore, an increase in the concentration of iron ions favors the formation of magnetite. Another very relevant variable in this reaction is pH: a basic pH boosts this process [17]. Thus, to ensure non-spontaneous conditions for magnetite synthesis, the reaction was performed at pH 2. To reinforce this hypothesis, the amyloid concentration was minimal, at a concentration thousand times lower than that of the iron compounds.

The formation of magnetite nanoparticles promoted by Aβ_{42} was studied by means of SEM, TEM, EELS and MFM. The lack of magnetite nanoparticle formation by spontaneous reaction in the absence of Aβ_{42} was tested with a control under the same conditions.

After the chemical reaction in the presence and absence of Aβ_{42}, particles of the magnetite–amyloid peptide complex were collected and concentrated with the help of an external magnet. The concentrated sample was left to dry on an electrically conductive double-sided adhesive carbon disk substrate, where it was studied by SEM (figure 1). SEM characterization allows the determination of differences in the topographies of the synthesized molecules.

In the absence of amyloid, bulk precipitates of iron hydroxide were formed. In contrast, monomeric Aβ_{42} was bound to the iron and oligomeric structures self-assembled into protofibrils and mature fibrils, initiating the mechanism of concentration of iron hydroxides entrapped inside the woolly ball-like structures of Aβ_{42} fibrils. A high iron

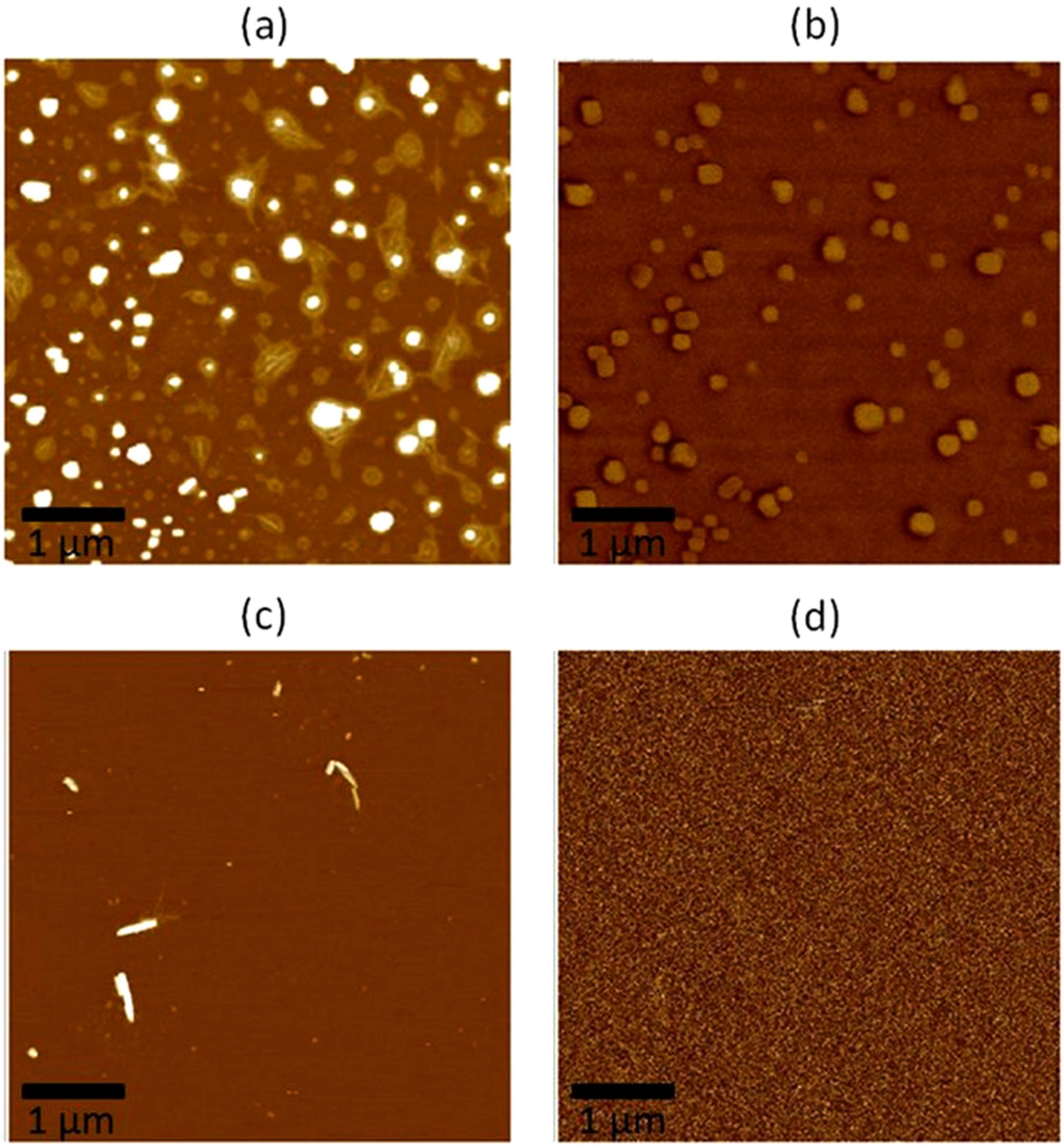

Figure 2. MFM topography image ($5 \times 5\ \mu m^2$) (a)–(c) and phase image (b)–(d) of nanoparticles in the presence (a), (b) and absence (c), (d) of Aβ_{42}. Scale bars $= 1\ \mu$m.

concentration tilts the magnetite reaction towards formation of these nanoparticles.

While SEM offers a picture of the nanoparticles formed and the fibrils of amyloid peptide growing around the particles, MFM provides a closer image with more information about the shape and structure of the molecules adsorbed on the surface. Moreover, the latter technique offers the possibility of highlighting the magnetic nanoparticles, thus distinguishing them from the non-magnetic ones and clearly differentiating the magnetite from other non-magnetic subproducts in the synthesis.

Figure 2 shows the topography of nanoparticles synthesized in the presence and absence of Aβ_{42}. As observed in the SEM characterization, clusters of nanoparticles were only detected in the samples synthesized with Aβ_{42} (figure 2(a)) (they are the clusters surrounded by Aβ_{42} fibrils, as already observed in the SEM results). The magnetic nature of the nanoparticles was confirmed in the MFM phase images (figure 2(b)), which only reproduce the shape of the magnetic nanoparticles observed in the topographic images, the diffuse image of amyloid being lost (figure 2(d)).

On the other hand, the topographic image of the sample without Aβ_{42} shows bulk precipitates of iron hydroxide (figure 2(c)). The non-magnetic nature of this aggregation is confirmed in the phase image, which is not detected by the magnetic tip of the MFM (figure 2(d)). These results reveal the promoting effect that amyloid peptide has on the synthesis of magnetic nanoparticles. The capacity of this peptide to

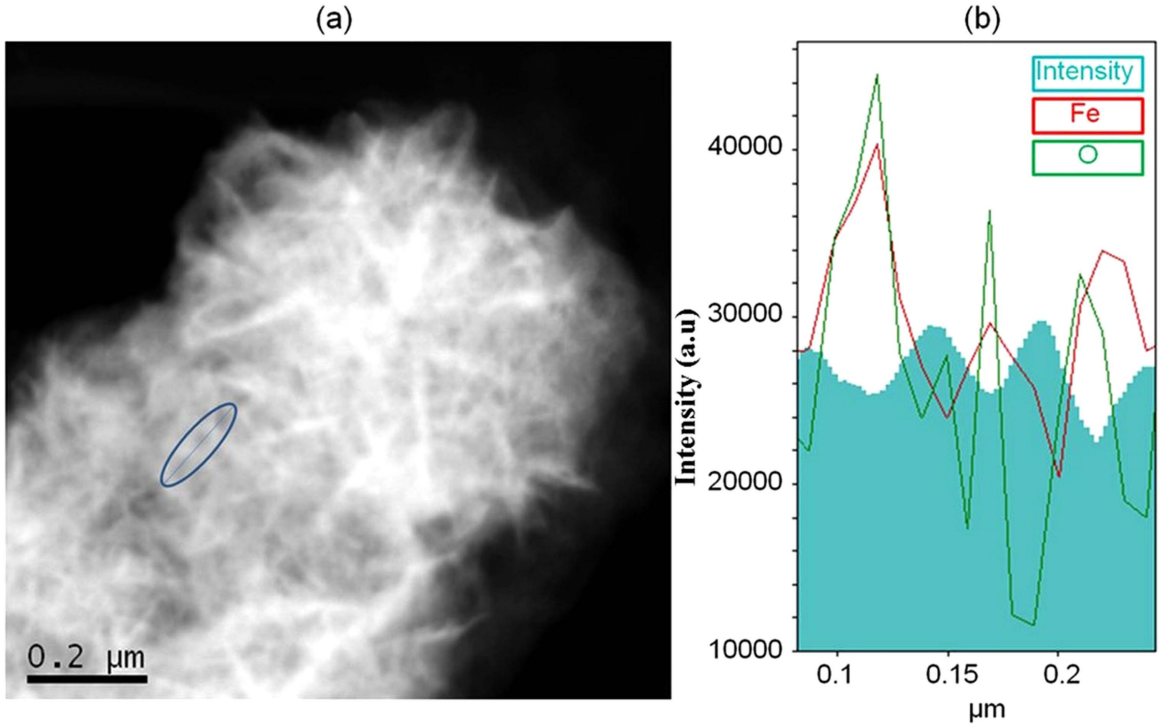

Figure 3. TEM images of an amyloid plaque with embedded magnetic nanoparticles (a) and EELS results for the selected zone with iron (Fe) and oxygen (O) intensity (b).

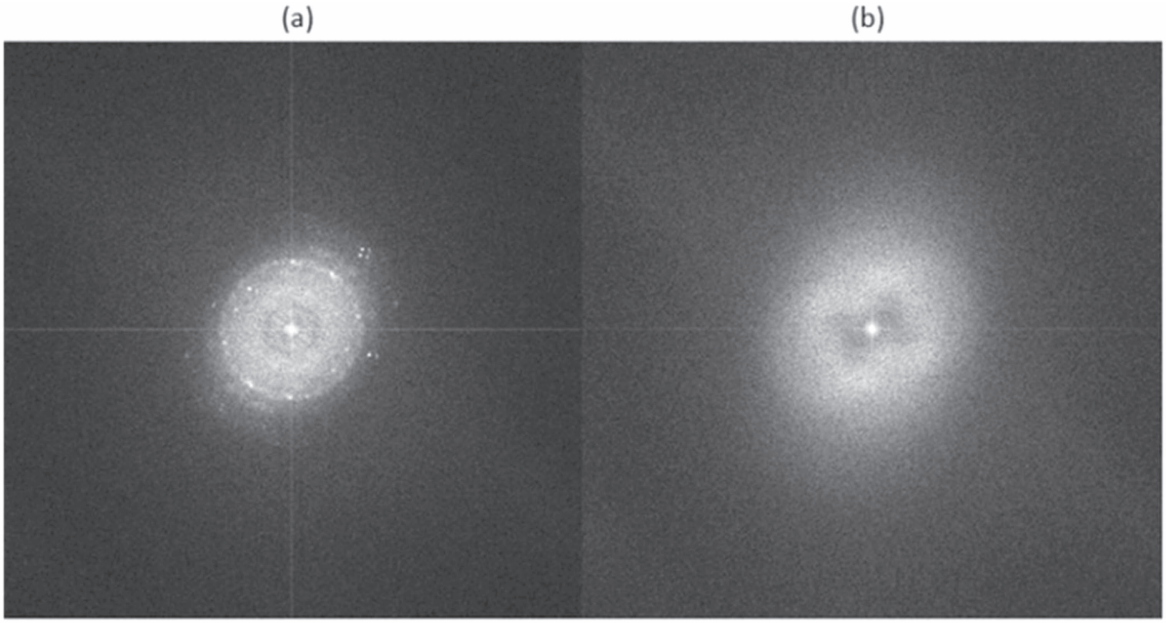

Figure 4. Electron diffraction images of polycrystalline magnetic nanoparticles (a) and amorphous structure of β-amyloid (b).

encapsulate iron ions into its fibrils provides an efficient environment for the spontaneous production of these magnetic nanoparticles, even under conditions that are not favorable for this process, as shown by the control performed without amyloid peptide.

The structure of the fibrils formed by Aβ_{42} and the chemical nature of the embedded magnetite nanoparticles were studied by mean of TEM and EELS. TEM showed the structure of the fibrils developed as a result of Aβ_{42} aggregation. This structure was similar to that reported for amyloid plaques in brain slices from AD patients [15]. Inside the woolly ball structure of the amyloid peptide, magnetite nanoparticles were synthesized and entrapped inside the plaques of Aβ_{42} fibrils, which are clearly delimited in the TEM picture (figure 3(a)). The size and chemical make-up of the dark dots inside the amyloid fibrils were analyzed using

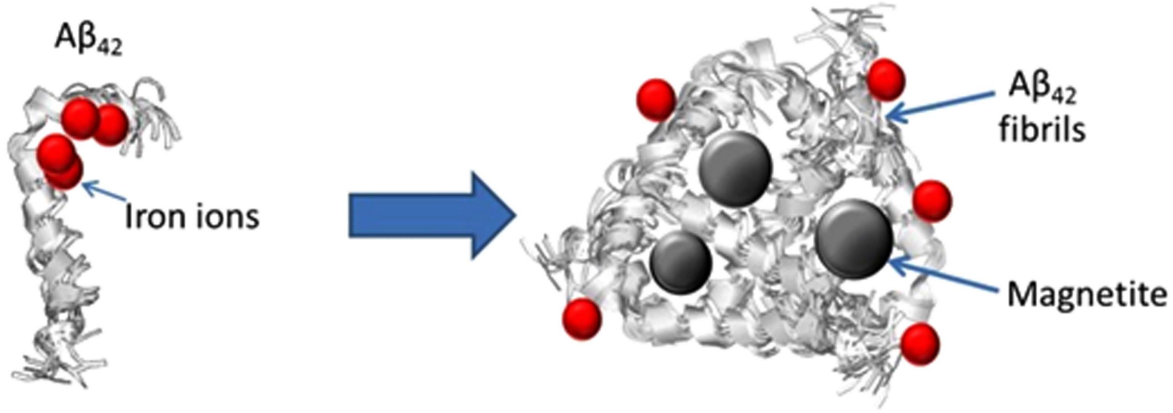

Figure 5. Schematic representation of magnetite formation by the entrapment of iron ions in amyloid fibrils.

Table 1. The *d*-spacing values from electron diffraction patterns (nm) for various planes of the polycrystalline structure of the nanoparticles entrapped in the amyloid fibrils (left column), and reported values of *d*-spacing with the x-ray diffraction (XRD) technique for various planes of magnetite reported in the literature (right column).

Measured *d*-spacing values	Magnetite *d*-spacing values reported [17]
2.91	2.97
2.50	2.53
2.1	2.1
1.67	1.71
1.58	1.61

EELS. Figure 3(b) illustrates the electron intensity transmitted through the sample, showing the appearance of the metallic and non-metallic areas.

This result shows the profile of the nanoparticles in the TEM picture in figure 3(a). The differences in the intensity of the iron and oxygen concentrations along the substrate gives a topographic picture of the nanoparticles and an approximate value of their diameter (about 25 nm). These findings coincide with those for previously reported amyloid plaques with magnetic nanoparticles in slices of hippocampus from AD brains, in which amyloid polymers trapped magnetic nanoparticles [18]. The nanoparticle size is consistent with the reported size of the magnetic nanoparticles in the plaque core material of AD brains [6]. EELS also allow us to unravel the chemical nature of the profiled nanoparticles, showing the content of oxygen (green line in figure 3(b)) and iron (red line in figure 3(b)).

The EELS results reveal the iron and oxygen content of these nanoparticles, while the MFM results show their magnetic nature. However, these characterizations do not give a complete picture of the structure of these nanoparticles, since there are various types of magnetic iron oxide, with similar iron and oxide intensities. Thus, to determine the type of iron oxide particles present, we examined their crystalline structure using electron diffraction. This showed the polycrystalline structure of the entrapped nanoparticles (figure 4(a)). In contrast, an amorphous structure was observed in the fibrillar amyloid (figure 4(b)).

The planes of the nanoparticles' polycrystalline structure were studied by comparison with the tabulated values of *d*-spacing reported in the literature (table 1).

The *d*-spacing values for the planes of the polycrystalline structure of the nanoparticles synthesized in the presence of Aβ_{42} coincided with those reported in the literature for magnetite, thereby confirming that nanoparticles synthesized by the entrapment of amyloid peptide contained magnetite. This matched with the nanoparticle structure found entangled with amyloid peptide in AD brains.

The experimental results obtained agree with previous reports regarding the ability of amyloid to interact with and concentrate iron oxide [16]. The entrapment of a high concentration of magnetite precursors in a reduced volume leads to formation of iron oxide up to a critical concentration that tilts the balance of the magnetite synthesis reaction towards the formation of this nanoparticle (figure 5). In fact, similar behavior is reported in certain nanoparticle synthesis reactions, where a surfactant plays a multifunctional role—first coordinating and concentrating the initial particles to nucleate the synthesis of the nanoparticles for monodispersity [19].

4. Conclusion

In the present study, a key role for Aβ_{42} in magnetite formation is postulated. We demonstrated the generation of magnetite nanoparticles, under unfavorable pH conditions for their synthesis, only when iron was entrapped with Aβ_{42}, which was characterized with SEM, TEM, EELS and MFM. With the controls we successfully demonstrated that amyloid peptide promotes magnetite formation in the absence of ferritin protein. Magnetite nanoparticles were synthesized in an unfavorable environment for their spontaneous generation, but the presence of amyloid peptide permits the formation of these nanoparticles. The amyloid peptide appears to promote the synthesis of magnetite: the iron ions required for this synthesis are accumulated at a high concentration inside the fibrillar matrix of Aβ_{42}. In our conditions, although the low

pH hindered this reaction, the presence of Aβ_{42} and the local high concentration of trapped iron inside this peptide made the generation of magnetite nanoparticles feasible. Our results offer some answers regarding the biogenesis of magnetite found in amyloid plaques in AD patients and thus provide new insights into the pathogenesis of AD.

Acknowledgments

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References

[1] Davidson R G 2002 *PDQ Medical Genetics* vol 2 (Hamilton, ON: BC Decker) p 37
[2] Kuo Y M *et al* 1996 *J. Biol. Chem.* **271** 4077
[3] Gaasch J A, Geldenhuys W J, Lockman P R, Allen D D and Van der Schyf C J 2007 *Neurochem. Res.* **32** 1686
[4] Inglese M and Petracca M 2013 *Prion* **7** 47
[5] Teller S, Tahirbegi B I, Mir M, Samitier J and Soriano J 2015 *Sci. Rep.* **5** 17261
[6] Pankhurst Q, Hautot D, Khan N and Dobson J 2008 *J. Alzheimer's Dis.* **13** 49–52
[7] Roberts B R, Ryan T M, Bush A, Masters C L and Duce J A 2012 *J. Neurochem.* **120** 149
[8] Mir M, Tahirbegi I B, Valle-Delgado J J, Fernàndez-Busquets X and Samitier J 2012 *Nanomed. Nanotechnol. Biol. Med.* **8** 974
[9] Gang L, Garrett M, Men P, Zhu X, Perry G and Smith M A 2005 *Biochim. Biophys. Acta* **1741** 246
[10] Roberts B L, Ryan T M, Bush A I, Masters C L and Duce J A 2012 *J. Neurochem.* **120** 149
[11] Hautot D, Pankhurst Q A, Khan N and Dobson J 2003 *Proc. R. Soc. Lond.* B **270** 62
[12] Fisher P, Götz M E, Danielczyk W, Gsell W and Riederer P 1997 *Life Sci.* **60** 2273
[13] Stine W B, Dahlgrem K N, Krafft G A and LaDu M J 2003 *J Biol Chem.* **278** 11612–22
[14] Ma M, Zhang Y, Guo Z and Gu N 2013 *Nanoscale Res. Lett.* **8** 1
[15] Collingwood J F *et al* 2008 *J. Alzheimer's Dis.* **14** 235–45
[16] Khan A, Dobson J P and Exley C 2006 *Free Radic. Biol. Med.* **40** 557
[17] Faiyas A, Vinod E, Joseph J, Ganesan R and Pandey R K 2010 *J. Magn. Magn. Mater.* **322** 400
[18] Hautot D, Pankhurst Q A, Khan N and Dobson J 2003 *Proc. R. Soc. Lond.* B **270** S62
[19] Ott S, Dziadulewicz N and Crowther D C 2015 *Dis. Model. Mech.* **8** 657