Electrochemistry of Alzheimer Disease Amyloid Beta Peptides

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Abstract: Alzheimer’s disease (AD) is a widespread form of dementia that is estimated to affect 44.4 million people worldwide. AD pathology is closely related to the accumulation of amyloid beta (Aβ) peptides in fibrils and plaques, the small oligomeric intermediate species formed during the Aβ peptides aggregation presenting the highest neurotoxicity.

This review discusses the recent advances on the Aβ peptides electrochemical characterization. The Aβ peptides oxidation at a glassy carbon electrode occurs in one or two steps, depending on the amino acid sequence, length and content. The first electron transfer reaction corresponds to the tyrosine Tyr 10 amino acid residue oxidation, and the second to all three histidine (His 6, His 13 and His 14) and one methionine (Met 35) amino acid residues. The Aβ peptides aggregation and amyloid fibril formation are electrochemically detected via the electroactive amino acids oxidation peak currents decrease that occurs in a time dependent manner. The Aβ peptides redox behaviour is correlated with changes in the adsorption morphology from initially random coiled structures, corresponding to the Aβ peptide monomers in random coil or in α-helix conformations, to aggregates, protofibrils and two types of fibrils, corresponding to the Aβ peptides in a β-sheet configuration, observed by atomic force microscopy. Electrochemical studies of Aβ peptides aggregation, mediated by the interaction with metal ions, particularly zinc, copper and iron, and different methodologies concerning the detection of Aβ peptide biomarkers of AD in biological fluids, using electrochemical biosensors, are also discussed.

Keywords: Alzheimer’s disease (AD), amyloid beta (Aβ) peptide, voltammetry, fibrilization, aggregation, adsorption, oxidation, AD biomarkers.

1. INTRODUCTION

On November 3, 1906, at the Southwest German Psychiatrists meeting held in Tubingen, Alois Alzheimer reported for the first time, the Auguste Deter brain autopsy results. The findings showed in detail the histopathological features that are today associated with Alzheimer’s disease (AD): the massive loss of neurons and the presence of amyloid plaques and neurofibrillary tangles [1]. One year later, Alzheimer’s findings regarding the first patient identified with AD were summarised in a short communication [2]. Several years later, in 1911, in a much more detailed paper, Alzheimer discussed the concept of the disease in detail, presenting the case of Johann F, the second Alzheimer’s patient suffering from AD [3,4].

However, these reports did not attract too much attention, and only in 1984, the first study about the amyloid beta (Aβ) peptide purification and characterization appeared [5]. Shortly after, in 1991, the theory of ‘amyloid hypothesis’ was postulated by Selkoe [6], claiming that the neurodegeneration in AD was caused by the deposition of Aβ peptide plaques in the brain tissue [6, 7]. From that moment onwards, the scientific community gave a special attention to the Aβ peptide aggregation in fibrils and plaques and to the inhibition of this process. However, nowadays, the senile plaques are not anymore considered responsible for neurodegeneration, being the small oligomeric intermediate species that appear during the aggregation
process, from soluble monomers to protofibrils and fibrils, considered to induce toxicity, via chemical generation of reactive oxygen species [8,9].

Native human Aβ peptides are short ~ 4 kDa isoforms, varying from 39 to 43 amino acids, the predominant isoforms being the Aβ1-40 (85-90 %) and the faster-aggregating Aβ1-42 (10-15 %), Scheme 1. They are generated as soluble monomers, in response to injury, after the proteolytic cleavage of the transmembrane amyloid precursor protein (APP) by the proteases β- and γ-secretase, Scheme 1 [10-13]. In the normal brain, the Aβ monomers provide neuroprotection against oxidative stress, after which they are cleared into the extracellular matrix and can be found in the cerebrospinal fluid (CSF) in concentration in the nM range [14]. In the AD brain, dysregulation and overproduction of Aβ monomers occur, followed by a fibrillization process that involves numerous intermediate structures.

Different Aβ peptide sequences and their fibrillization process have been investigated by a wide range of standard techniques [15]: (i) electron microscopy, atomic force microscopy (AFM) and circular dichroism, for the Aβ peptides structural and morphological characterisation, (ii) size exclusion chromatography, light scattering and analytic ultracentrifugation, for the Aβ peptide molecular weight determination, (iii) fluorescence microscopy, thioflavin T (ThT) and Congo red (CR) assays, for studying the aggregation and inhibition mechanisms, and (iv) sandwich ELISAs, Bio-Barcode and immuno-fluorescent assays, for the selective identification and quantification of different Aβ peptide sequences aggregation.

The use of electrochemical methods only started at the beginning of the last decade, and add a very important and new perspective to the Aβ peptide research, due to their Aβ peptide amino acids oxidation mechanistic information, high sensitivity, simplicity, rapid response and compatibility with miniaturization.

In this review, the recent studies concerning the electrochemical characterisation of the Aβ peptides redox behaviour, fibrillization, interaction with metal ions, as well as electrochemical biosensors applications for the detection of Aβ peptides AD biomarkers, are revised.

2. Aβ PEPTIDES REDOX BEHAVIOUR

The Aβ peptides participate in redox reactions in the extra- and intra-cellular medium, therefore, understanding the Aβ peptide oxidative behaviour is crucial for understanding the properties of different Aβ peptide isoforms and their fibrillation mechanisms.

Among the 20 amino acids present in peptides and proteins, in aqueous solution, only five are

![Scheme 1](image_url)

Scheme 1. (A) Primary and (B) secondary structures [22,23] of human amyloid beta (Aβ) isoform peptides Aβ1-40 and Aβ1-42. Red dots mark the electroactive amino acid residues. [Adapted from Refs. [13,24] with permission].
electroactive: tyrosine (Tyr) [16-18], tryptophan (Trp) [18,19], histidine (His) [20], methionine (Met) [21] and cysteine (Cys) [21], and the oxidation peak currents relationship is $I_{\text{Tyr/Trp}} > I_{\text{His}} > I_{\text{Cys}} > I_{\text{Met}}$ [16, 20, 21].

The free Tyr amino acid undergoes a pH-dependent irreversible oxidation at glassy carbon electrode (GCE) and boron-doped diamond electrode (BDDE), that occurs at the hydroxyl group at $E_p \sim +0.65$ V and involves the formation of a thermodynamically unstable radical, followed by the formation of an electroactive orthoquinone, which is reversibly reduced to catechol in a two-electron two-proton mechanism [16-18,20], Fig. 1A.

The free Trp amino acid oxidation occurs in a pH-dependent, irreversible, two oxidation steps, the first step corresponding to the pyrrole ring oxidation and the second one to the indole electrochemical hydroxylation of the benzene moiety, Fig. 1A. The second step leads to the formation of two electroactive products, which adsorb on the electrode surface and are further reversibly oxidized [18-20]. At the GCE, the Trp first oxidation peak potential is similar to the Tyr oxidation potential at $E_p \sim +0.65$ V, Fig. 1A, while at BDDE, the Trp first oxidation peak occurs well separated from the Tyr oxidation peak at $E_p \sim +0.75$ V. Moreover, the Trp second oxidation peak at the BDDE, occurs at a more positive potential, $E_p \sim +1.10$ V, than at the GCE, at $E_p \sim +1.02$ V.

The free His amino acid undergoes a pH-dependent irreversible oxidation, at GCE and BDDE, that occurs in a single step, at $E_p \sim +1.10$ V in pH 7.0, Fig. 1A. In the pH range 6.0 < pH < 9.0, the His carboxyl group is deprotonated, while the amino group is protonated and the imidazole ring is neutral. The oxidation of the imidazole group occurs only between these pHs, in a mechanism involving the transfer of one electron and one proton, with the formation of 2-oxo-histidine.

The free Met amino acid oxidation at GCE is irreversible, diffusion-controlled, pH-dependent and occurs in two consecutive steps, Fig. 1A, involving, the first step, at $E_p \sim +1.0$ V, the thioether group oxidation and the sulfoxide formation, and the second step, at $E_p \sim +1.23$ V, the sulfone formation [21]. At the BDDE, free Met undergoes oxidation in only one-step, due to a negligible adsorption and the oxidation mechanism corresponds to the formation of methionine sulfoxide.

The free Cys amino acid oxidation at the GCE and BDDE is an irreversible, diffusion-controlled pH-dependent process and occurs in a complex mechanism [21]. The first step, at $E_p \sim +0.55$ V, involves the oxidation of the sulfhydryl group and the formation of a disulfide bridge between two Cys moieties, with the formation of cystine, followed by the oxidation of cystine at a higher potential, Fig. 1A.

The peptides and proteins oxidation, at carbon electrodes, in aqueous solution, take place only at these five electroactive Tyr, Trp, His, Met and Cys amino acid residues, Fig. 1B [20]. At the GCE, the Trp first
The Aβ peptides play a critical role in the AD pathogenesis, the most important being Aβ1-40 and Aβ1-42 peptide sequences, since they are present in more than 90% of amyloid plaques. Structurally Aβ1-40 and Aβ1-42 contain three electroactive amino acid residues: one tyrosine (Tyr10), three histidine (His6, His13 and His14) and one methionine (Met35), Scheme 1A. The amino acid residues distribution makes each Aβ peptides chain unique, from the point view of structure, physicochemical properties and function. The Aβ1-40 and Aβ1-42 peptides three dimensional structures, Scheme 1B, present a mixed random coil and α-helical structures for Aβ1-40, and an unstructured random coil structure for Aβ1-42. The position of the electroactive amino acids Tyr, His and Met in the peptide chain, and the peptide secondary structure is very important, when studying the Aβ peptide electrochemical oxidation. The Tyr10 and His13 amino acid residues are more accessible to the electrode surface enabling their easier oxidation, whereas the His6, His14 and Met35 amino acid residues are less accessible and more difficult to be oxidized, Scheme 1B.

The Aβ1-40 and Aβ1-42 peptides voltammetric behaviour, at GCE, dependent on the concentration and adsorption time, has been investigated [24]. DP voltammograms recorded, in pH 7.4, in freshly prepared Aβ1-40 and Aβ1-42 peptide solutions, Fig. (2A→), and at Aβ1-40 and Aβ1-42 peptide films adsorbed at GCE, Fig. (2B→), always showed the occurrence of two consecutive oxidation peaks. The first oxidation peak corresponds to the Tyr10 amino acid residue oxidation, at \( E_p \sim +0.65 \text{V} \), while the second oxidation peak corresponds to the three histidine, His6, His13 and His14 and one methionine, Met35, amino acid residues oxidation, at \( E_p \sim +1.00 \text{V} \) [24].

A qualitative electroanalytical investigation of the oxidative behaviour of specifically selected twelve Aβ peptide sequences, human Aβ1-40 and Aβ1-42, inverse Aβ40-1 and Aβ42-1, fragments Aβ1-28, Aβ1-16, Aβ10-20, Aβ12-28, Aβ17-42, mutants Aβ1-40Phe10 and Aβ1-40Nle35, and rat Aβ1-40Rat, has been performed, Scheme 2 [24]. The goal was to identify the electroactive amino acid residues of the Aβ monomers, before the aggregation process started, which may be involved in the Aβ peptide electron transfer biochemical oxidation reaction. Depending on the amino acid sequence, length and content, the DP voltammograms obtained in Aβ peptide solutions or at Aβ peptide films adsorbed at GCE, showed the occurrence of one or two oxidation peaks, Figs. (3-5). The first electron transfer reaction corresponds to the Tyr10 residue oxidation, and the second to the His6, His13, His14 and Met35 amino acid residues oxidation. The highest contribution to the second oxidation peak current was from His13 residue, followed by His14 and His6 residues, while the Met35 residue had the lowest contribution. The DP voltammetric results showed that the Aβ peptides electron transfer depends on the peptide hydrophobicity, structure, and the position of the electroactive amino acid residues in the sequence, the residues close to N-termini giving the highest oxidation peak currents [24].

The adsorbed Aβ1-40 and Aβ1-42 peptide sequences conformation on GCE present the Tyr10 and His13 amino acid residues more accessible to the GCE surface, enabling their easier oxidation, whereas His6, His14 and Met35 are less accessible and more difficult to be oxidized, Scheme 1 and Fig. (3) [24]. The highest oxidation peak currents occurred for Aβ10-20 peptide (Tyr10 near N-termini, His13, His14), and for the

![Scheme 2](image)

Scheme 2 - The Aβ isoform peptides: human Aβ1-40 and Aβ1-42, mutants Aβ1-40Phe10 and Aβ1-40Nle35, and Aβ1-40Rat. The electroactive amino acid residues are underlined. [Adapted from Ref. [13] with permission].

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hydrophobic peptide sequences $\text{A}^\beta_{12-28}$ (His$^{13}$ near N-termini, His$^{14}$) and $\text{A}^\beta_{1-28}$ (His$^6$, Tyr$^{10}$, His$^{13}$, His$^{14}$), which contained the electroactive amino acid residues in the middle of the chain, Fig. (4) [24]. Small oxidation peak currents were obtained for the short $\text{A}^\beta_{1-16}$ peptide (His$^6$, Tyr$^{10}$, His$^{13}$ and His$^{14}$ near C-termini), which was less hydrophobic, and for $\text{A}^\beta_{17-42}$ peptide (Met$^{35}$), where Met$^{35}$ was the only electroactive amino acid.

![Fig. (2). DP voltammograms baseline corrected at GCE, in pH 7.4: (▬) 50 µM $\text{A}^\beta_{1-40}$ and $\text{A}^\beta_{1-42}$ peptides freshly prepared solutions and (▬) $\text{A}^\beta_{1-40}$ and $\text{A}^\beta_{1-42}$ peptide adsorbed at GCE. [Adapted from Ref. [24] with permission].](image1)

![Fig. (3). DP voltammograms base line corrected in pH 7.4, of $\text{A}^\beta_{1-40}$, inverse $\text{A}^\beta_{40-1}$, $\text{A}^\beta_{1-28}$, $\text{A}^\beta_{1-16}$, $\text{A}^\beta_{10-20}$, $\text{A}^\beta_{12-28}$, and $\text{A}^\beta_{17-42}$ peptides adsorbed at GCE. [Adapted from Ref. [24] with permission].](image2)

In the mutant $\text{A}^\beta_{1-40}\text{Phe}^{10}$ peptide, the tyrosine Tyr$^{10}$ amino acid residue is substituted by phenylalanine Phe$^{10}$, and in the mutant $\text{A}^\beta_{1-40}\text{Nle}^{35}$ peptide, the methionine Met$^{35}$ is substituted by norleucine Nle$^{35}$, Scheme 2, and DP voltammetric results showed that even the substitution of a single non-electroactive amino acid induced changes on the oxidative behaviour, Fig. (5) [24].

In the $\text{A}^\beta_{1-40}\text{Rat}$ peptide, three amino acids are substituted: Arg$^5$ by Gly$^5$, Tyr$^{10}$ by Phe$^{10}$ and His$^{13}$ by
Arg\textsuperscript{13} Scheme 2. These substitutions, with the neighbours Gly\textsuperscript{5} near His\textsuperscript{5} and Arg\textsuperscript{13} near His\textsuperscript{14}, affect the electroactive His susceptibility for oxidation, when compared with the human Aβ\textsubscript{1-40} peptide. The Aβ\textsubscript{1-40}Rat peptide has only three, His\textsuperscript{6}, His\textsuperscript{14} and Met\textsuperscript{35}, electroactive amino acid residues compared with human Aβ\textsubscript{1-40} with five, His\textsuperscript{6}, Tyr\textsuperscript{10}, His\textsuperscript{13}, His\textsuperscript{14} and Met\textsuperscript{35}, electroactive amino acid residues, but the second oxidation peak current was similar, Fig. (5).

![Fig. (5). DP voltammograms base line corrected in pH 7.4, of human Aβ\textsubscript{1-40}, and mutant Aβ\textsubscript{1-40}Phe\textsuperscript{10}, and Aβ\textsubscript{1-40}Nle\textsuperscript{35} and Aβ\textsubscript{1-40}Rat peptides adsorbed at GCE. [Adapted from Ref. [24] with permission].](image-url)

The fact that rats are not affected by AD and the differences observed between the oxidative behaviour of these peptides highlights the specific importance of Tyr\textsuperscript{10} and His\textsuperscript{13} electroactive amino acid residues on the secondary structure, and the fact that these residues may be active centres of toxic species linked to neurodegeneration [24].

The direct electrochemical oxidation of synthetic Aβ peptides has also been investigated by the square wave (SW) voltammetry at screen printed carbon electrode (SPCE) [25, 26]. The correspondence between the Aβ\textsubscript{1-42} oxidation peaks and the amino acid residues has been established based on the analysis of the SW voltammograms obtained for free Tyr, His, Met amino acids, the Aβ\textsubscript{1-16} peptide (representing the Aβ metal-binding domain and lacking the Met residue), Aβ\textsubscript{1-16} peptide mutants with different number of His residues and the rat Aβ\textsubscript{1-16} peptide sequence lacking the Tyr residue [25]. The results showed that the Aβ peptides oxidation, followed via the His residues oxidation peak, may serve as an indicator of the metal ion-Aβ complex formation.

The oxidation of the Aβ\textsubscript{1-16} peptide, the human Aβ peptide metal-binding domain, Scheme 2, by cyclic voltammetry (CV) and SW voltammetry at SPCE, has been investigated [27]. The Aβ\textsubscript{1-16} peptide Tyr\textsuperscript{10} amino acid residue oxidation peak potential was compared with the free Tyr amino acid and the Tyr-lacking Aβ\textsubscript{1-16}Rat peptide oxidation peak potentials.

The Aβ\textsubscript{1-42} peptide, its isomers with familial mutations (D7H or H6R) and the naturally occurring modification (Ser-8 phosphorylation) have been investigated by CV and SW voltammetry, at SPCE [26]. For all peptides, the voltammograms showed an anodic peak corresponding to the Tyr\textsuperscript{10} oxidation, a small peak corresponding to the His\textsuperscript{6}, His\textsuperscript{13} and His\textsuperscript{14} oxidation, and a large wave corresponding to the Met\textsuperscript{35} amino acid residue oxidation. To confirm the oxidation of Met\textsuperscript{35} in Aβ\textsubscript{1-42} peptides, the short peptide Aβ\textsubscript{1-16}, lacking the Met, was employed. The Tyr\textsuperscript{10} amino acid residue oxidation peak analysis, based on the potential and current changes, allowed to discriminate the Aβ\textsubscript{1-42} peptide from some of its isoforms [26].

The Aβ peptides and insulin are both amyloidogenic peptides, sharing a common sequence recognition motif. They are substrates for the same insulin degrading enzyme, and the AD pathology is also characterised by an impaired glucose metabolism [28].

The electrochemical oxidation of insulin amyloids at GCE by CV and DP voltammetry, was studied and compared with the model polypeptide poly(l-tyrosine), the simplest Tyr-rich polypeptide is capable to form β-sheet conformations [29]. The peptides redox behaviour was determined by the polypeptide chains conformation, dependent on the Tyr residues accessibility for the oxidation in each structure. Similarities in the electrochemical behaviour of insulin amyloid and poly(L-Tyr) film deposited from NH\textsubscript{3} aq, were interpreted in terms of similar β-sheet structures formed and confirmed by FTIR. The poly(L-Tyr) films deposited from THF showed no β-sheet formation in the FTIR spectra and different electrochemical behaviour was observed. This was explained in terms of better electrochemical accessibility of Tyr residues in well-ordered, β-sheet films as compared to random coil structures obtained in THF solutions [29].
3. Aβ PEPTIDES FIBRILIZATION PROCESS

In AD brain, the Aβ peptides dysregulation and overproduction lead to amyloid fibril formation, fibrillization or amyloidogenesis, a process that occurs through endogenous native Aβ peptides self-assembling into highly ordered aggregates with cross-β-sheet rich structures. The Aβ peptides morphological changes from soluble, monomeric random coil or α-helix conformations into the aggregated β-sheet structures, involve the formation of numerous intermediates, including low-molecular weight structures (e.g. dimers, trimers, and tetramers), globular oligomers and protofibrils. Since 2005, the direct Aβ peptides electrochemistry also started to be used for monitoring Aβ fibrillation, complementary to other aggregates detection techniques normally employed.

The first report on the electrochemical detection, characterization and kinetic study of the Aβ₁₄₀ and Aβ₁₄₂ peptide aggregation was based on detecting the changes in only the Tyr¹⁰ amino acid residue oxidation peak, at GCE [30]. As the Aβ peptide aggregate, structural and conformational changes occur, which affect the degree of Tyr¹⁰ amino acid residues exposure to the electrode surface. The voltammetric results showed significant differences in the aggregation process between the two peptide sequences, confirmed by fluorescent and AFM studies [30].

AFM and DP voltammetry were also used to determine successive stages of Aβ₁₄₂ peptide aggregation and amyloid fibril formation, via Tyr¹⁰ residues oxidation at HOPG [31]. The adsorbed Aβ peptides morphological changes were correlated with their redox behaviour. Based on the Tyr¹⁰ residues oxidation, a quantitative estimation of the Aβ₁₄₂ peptide aggregation in vitro, at the surface of SPCE, has been reported [32]. The Tyr¹⁰ oxidation peak current of the Aβ₁₄₂ peptide in different aggregation states was directly compared with the size and structure of the Aβ₁₄₂ peptide aggregates, estimated by dynamic light scattering and ThT-based fluorescence. The same electrochemical procedure was applied for the Aβ₁₄₂ peptide, at the hydrophilic surface of spectroscopic graphite [33], the Tyr¹⁰ amino acid residues electrochemical oxidation allowing the discrimination between the different stages of the adsorbed Aβ₁₄₂ peptide.

The aggregation of the Aβ₁₄₀ and Aβ₁₄₂ peptides, immobilised onto a saccharide layer, by DP voltammetry, has been detected [34]. The densely-packed saccharide area was fabricated onto a carbon electrode by three steps: electrochemical deposition of gold nanoparticles (AuNPs) on a screen printed strip, formation of an acetylenyl group self-assembled monolayer onto the AuNPs and the cycloaddition of an azide-terminated sialic acid to the acetylenyl group. The attachment of the Aβ peptides to the sialic acid layer was confirmed by electrochemistry and AFM. The Tyr¹⁰ amino acid oxidation peak current was dependent on the Aβ₁₄₀ and Aβ₁₄₂ peptides concentration.

The Tyr¹⁰ amino acid residue oxidation, mediated by tris-(2,2′-Bipyridine) osmium(II) chloride, has been used to detect the Aβ₁₄₀ peptides aggregation [35]. By means of SW voltammetry, it was possible to differentiate between Aβ peptides containing equimolar Tyr concentrations, and species with sizes from monomeric to insoluble aggregates were detected.

The inhibition of the Aβ₁₄₂ peptide aggregation and the disaggregation effect caused by a β-sheet breaker, have been studied by CV and DP voltammetry, and compared with Thioflavin-T-induced fluorescence, gel electrophoresis and electron microscopy [36]. The Tyr¹⁰ amino acid residue oxidation was followed at carbon nanotubes (CNTs) modified GCE immersed directly in the Aβ₁₄₂ peptide solution. The results provided new insights concerning the disposition of the Aβ₁₄₂ peptide N-terminal residue, in the structure of small aggregates, fibrils and amorphous aggregates. At the early stage of aggregation, it was observed that Tyr amino acid residue is available for oxidation, and during fibrillization, the oxidation peak disappears, showing that the unstructured N-terminal domain is only 9 amino acids long or even shorter [36].

The investigation of both Aβ peptides oxidation peaks, the first corresponding to the Tyr amino acid residues oxidation and the second, at higher potential, corresponding to the three His and Met amino acid residues oxidation, permitted a better correlation between the morphological modifications that occur during the fibrillization process and the changes of the Aβ peptides redox behaviour [13]. The time dependent structural modifications undergone by the human Aβ₁₄₀ and Aβ₁₄₂ peptides were investigated, by DP voltammetry at GCE and AFM at HOPG [13]. The Aβ₁₄₀ and Aβ₁₄₂ peptides intermediary structures which appear during fibrillization were compared with those obtained for five control peptide sequences (inverse Aβ₁₄₀ and Aβ₁₄₂, mutant Aβ₁₄₀Phe¹⁰ and Aβ₁₄₀Nle³⁵, and rat Aβ₁₄₀Rat). Lower aggregation rates were promoted by incubating the Aβ peptide solutions at room temperature and in free chloride media.
The Aβ1-40 and Aβ1-42 peptides aggregation, from soluble monomers to fully formed fibrils through intermediate structures, revealed to be a sequence-structure process, dependent on the physicochemical properties of each Aβ peptide isoforms, occurring at different rates and by different pathways [37,38]. The fibrilization process was followed by AFM, via changes in the adsorption morphology from: (i) initially random coiled structures of ~ 0.6 nm height, corresponding to the Aβ peptide monomers in random coil or in α-helix conformations, to (ii) aggregates and protofibrils of 1.5 - 6.0 nm height, and (iii) two types of fibrils, corresponding to the Aβ peptide in a β-sheet configuration, Figs. (6-8) [13]. The fibrillization was followed by DP voltammetry, via the time-dependent decrease and disappearance of both oxidation peak currents of the electroactive residues, corresponding to the Tyr10 amino acid residue oxidation, the first peak and to the three His6, His13, His14 and Met35 residues amino acid oxidation, the second peak, Figs. (8 and 9), and compared at each particular time with the morphological structures observed by AFM, Figs. (6 and 7).

Using control sequences that: (i) do not aggregate (inverse Aβ40-1 and Aβ42-1 peptides, and Aβ1-40Rat peptide) and (ii) Aβ peptides specially designed to lack specific electroactive amino acids (mutant Aβ1-40Phe10 and Aβ1-40Nle35 peptides), it was demonstrated that the decrease of the Aβ peptides oxidation peak currents is effectively due to aggregation and not due to another process. All these control Aβ peptide sequences do not aggregate, no protofibriles or fibrils were observed, and the oxidation peak currents did not change, by increasing the incubation time from 24 h to 48 h, Figs. (9 and 10) [13].

Closer attention was paid to the carbon electrode surface reactivity, which directly influenced the formation of fibrils at the electrode surface. AFM images showed differences between fibrils formed in solution or immobilised onto the carbon surface. The fibrils formed onto HOPG were thin, very reactive and presented a beaded morphology due to the adsorption of Aβ peptide aggregates onto their structure, Fig. (7C-ii), while the fibrils formed in solution, grown by attaching Aβ peptide monomer units to the end of the fibril, were long, continuous and twisted together into helices, Fig. (7C-iii) [13].

Electrochemical studies of Aβ peptides aggregation, using redox mediators, particularly redox dyes, such as CR diazo dye [39], ThT and BTA-1 benzothiazole dyes [40], commonly used for Aβ plaques staining, have been reported.

CR diazo dye electrochemistry has been used to monitor the Aβ peptides aggregation kinetics on disposable SPCEs [39]. The DP voltammetric results showed that the formation of a stable Aβ oligomeric species took place after ~400 min in vitro, in agreement with the spectroscopic results, proving that the CR diazo dye equimolar concentrations does not change the rate of aggregation.

Fig. (6). AFM images of Aβ1-40 peptides at different incubation times, adsorbed onto HOPG, from solutions of (A-C) 1.0 µM and (D-F) 10 µM Aβ1-40 peptides in pH 7.4. Each scale bar represented 100 nm. [Adapted from Ref. [13] with permission].
Concerning the benzothiazole dyes, ThT dye is positively charged and water-soluble, whereas BTA-1 is neutral and hydrophobic, both intercalating between the peptides β-sheets. The interfacial properties of the Aβ1-40 and Aβ1-42 peptides, and the course of their aggregation in vitro, in the presence of ThT and BTA-1, have been described [40]. The Aβ1-40 aggregation in the presence of BTA-1 led to a decrease of the BTA-1 oxidation peak current, while the incubation of Aβ1-42 peptide with BTA-1 led to an increased rate of exponential decay, which was in agreement with the Aβ1-42 peptide faster aggregation. The Aβ1-40 and Aβ1-42 peptides aggregation in the presence of ThT resulted in an increase of the ThT oxidation peak current after 24 h incubation.

The very early changes in the interfacial behaviour of the Aβ peptides after the first few minutes of
incubation were attributed to their fast oligomerization, with the disruption of the intercalative properties of the benzothiazole dyes between the β-sheets. The subsequent changes in the electrochemical currents were related to the onset of intercalation between the fibrils [40].

4. Aβ PEPTIDES INTERACTION WITH METAL IONS

The AD is characterized by both the deposition of amyloid plaques, constituted mainly by aggregated Aβ peptides, in the brain, and by obvious oxidative damage, including glycation, protein, DNA and RNA oxidation and lipids peroxidation [41]. The Aβ peptides aggregation is mediated by the interaction with metals, particularly zinc (Zn) [42], copper (Cu) [43] and iron (Fe) [44, 45]. Although an altered metal homeostasis is considered an important factor leading to AD, the exact role of metal ions in β-sheet formation and Aβ peptides fibril formation in vivo is still unclear.

The Aβ peptide direct electrochemistry based on the oxidation of a Tyr amino acid residue has been
employed to study the Aβ peptide conformational changes after the interaction with different concentrations of Zn(II) ions, within the pH range from 5.5 to 9 [46]. The Zn(II) ions binding to the Aβ1-16 peptide (the human Aβ peptide metal-binding domain, Scheme 2), and to various Aβ peptides isoforms with amino acid substitutions and post-translational modification (D7H, D7N, H6R, H6A-H13A, E11K, and pS8), by SW voltammetry, at SPCE, have been studied [46]. Except for H6A-H13A-Aβ1-16 peptide, addition of Zn(II) ions significantly decreased, and shifted to more positive potentials the Tyr10 amino acid residue oxidation peak of the Aβ1-16 peptide and its isoforms. The H6A-H13A-Aβ1-16 peptide showed different electrochemical behaviour, both in the absence and presence of Zn(II) ions.

The Zn(II), Cu(II), Mg(II) and Ca(II) ions interaction with the Aβ1-16 peptide within a wide range of ion concentrations in 5 < pH < 9, have also been studied [27]. Addition of both Zn(II) and Cu(II) ions significantly reduced the Tyr10 amino acid residue oxidation peak current and shifted the peak to more positive potentials, while Mg(II) and Ca(II) ions showed no noticeable effect [27].

The Aβ peptides aggregation kinetics in the absence/presence of Zn(II), Cu(II) and Fe(II) ions, by monitoring the Tyr10 oxidation peak current modifications, have been investigated [47]. In order to determine the influence of the metal chelating His-rich region and of the Aβ peptide hydrophobic region, a comparison between the full length Aβ1-42 peptide and the truncated 16 amino-acid Aβ1-16 peptide sequence, has been performed. The SW voltammetric results showed that Fe(II) and Cu(II) increase the Aβ peptide aggregation rate to a great extent, and that the Aβ1-42 hydrophobic region, which is not found in Aβ1-16, is important in the formation of stable metal-induced Aβ aggregates [47]. Together with the transmission electron microscopy, SW voltammetry at GCE, has been used to study the metal-induced Aβ1-40 peptide aggregation, the aggregation kinetics and the morphology in the absence/presence of Zn (II) and Cu (II) [48].

The Aβ peptides catalyse the reduction and/or oxidation of the metal ions between different oxidation states, which in the absence of sufficient antioxidant mechanisms, can lead to the production of toxic reactive oxygen species (ROS) in vivo that may contribute to the AD pathogenesis [44, 49]. Therefore, the Aβ peptide-metal ion complexes are potentially critical for AD pathology.

In order to understand the role of Cu in the Aβ peptides electrochemical reactivity, the influence of Cu(I) and Cu(II) binding to the Aβ1-40 peptide secondary structure, hydrophobicity, redox potentials and oxidation product formation, has been investigated [50]. The results showed that Cu(I) and Cu(II) complexation with the Aβ1-40 peptide reduce the metal electrochemical activity, the Cu(I)-Aβ1-40 and Cu(II)-Aβ1-40 complexes formation being associated with the redox potential modification. Fluorescent, circular dichroism, CV and DP voltammetry suggested that the redox current decrease could be due to the conformational changes that diminished the Cu accessibility to the external environment.

The binding stoichiometry between Cu(II) and the Aβ1-42, Aβ1-16 and Aβ1-28 peptides, by CV and electrospray ionization-Fourier transform ion cyclotron resonance mass spectrometry, has been determined [51]. The results showed that the Cu oxidation state within the resultant complexes was 2+, and the Tyr10 and Met35 amino acid residues were not oxidized in the Cu(II)-Aβ1-42 complex. Moreover, the Cu(I)-Aβ1-42 complex was electrochemically generated and was found to catalyse the reduction of O2 to produce H2O2 [51]. The Aβ1-42, Aβ1-16 and Aβ1-28 peptides voltammetric behaviour suggested that O2 diffusion to the metal centre can be affected by the Aβ peptide length and hydrophobicity. The Cu(II)-Aβ complex formed, in solution, was found by CV to be readily reduced by ascorbic acid. The H2O2 produced, in addition to its role in damaging DNA, protein and lipid molecules, can also be involved in further consumption of antioxidants, causing their depletion in neurons, and eventually damaging the neuronal defence system [51].

Two Cu(II)-Aβ1-16 peptide complexes that coexist at physiological pH, and reflect two different coordination modes (I and II), have been investigated, by CV, circular dichroism and electron paramagnetic resonance [52]. The coordination mode I predominates at pH ~ 6.7, and the coordination sphere involves two His residues, at positions 6 and 13 or 14, the N-terminal group and an oxygen-based ligand. The coordination mode II predominates at pH ~ 8.2, and involves a deprotonated amide group N– that replaces one of the His ligands, while the oxygen-based ligand is assigned to the backbone carbonyl group of Ala2. The results showed that the two Cu(II)-Aβ1-16 peptide complexes have distinct redox behaviour: coordination mode I has a reduction potential that allows it to be reduced and activate O2, while coordination mode II is redox-inactive under physiological conditions. The
existence of an intermediate Cu(I) species was determined [52]. The redox behaviour of the Aβ_{1-16} peptide in the two Cu(II)-Aβ_{1-16} peptide complexes, studied by CV, ascorbate consumption assay, and the consequent free radical reactions using mass spectrometry, showed that, in the presence of Cu(II), the Aβ_{1-16} peptide has higher propensity for H₂O₂ generation [53].

The Cu(II) complexes of Aβ peptides metal binding domains, Aβ_{1-16} and N-truncated Aβ_{4-16} containing a novel N-terminal FRH sequence, and shorter mutants were studied by CV [54]. The Aβ peptide sequence and Aβ peptide to Cu(II) molar ratio influence on the Cu(II)-Aβ_{4-x} complexes electrochemical properties, and the reversibility of the studied redox processes, have also been investigated. The results demonstrated the crucial role of Tyr^{10} amino acid residue in the redox process of the Cu(II)-Aβ_{4-x} peptide complex, leading to the irreversibility of the Cu(II)/Cu(III) redox couple [54].

The different Cu(II)-Aβ peptide complexes ability to generate hydroxyl radicals in the presence of ascorbate have been investigated, and the results were compared with other biological Cu-peptide complexes in order to get an insight into the biological relevance [55]. The Cu-Aβ peptide complexes produced more hydroxyl radicals than the complex of Cu with Asp-Ala-His-Lys (Cu-DAHK), but less than with Gly-His-Lys (Cu-GHK). By CV, it was found that the reduction potential relationship was Cu-GHK > Cu-Ab > Cu-DAHK, but the oxidation potential relation was the inverse, the reversible Cu redox cycling being correlated to higher hydroxyl radicals’ production. The Cu-Aβ_{1-42} complex showed a hydroxyl radical production five-times higher than the Cu-Aβ_{1-40} complex [55].

The kinetic and mechanistic studies of the ascorbic acid oxidation reaction by O₂, facilitated by Cu(II) complexes with Aβ_{1-16} and Aβ_{1-42} peptides, and Aβ_{1-42} aggregates, by CV, at GCE and compared with UV-VIS spectrophotometry, have been studied [56]. The reaction rate increased linearly with the Aβ-Cu(II) concentrations and dissolved O₂. The reaction stoichiometry between ascorbic acid and O₂ was 1:1 when the Aβ peptides concentration was much higher than Cu(II) concentration [56]. A mechanism for the ascorbic acid oxidation, in which the Cu oxidation states in the complex with the Aβ peptides alternated between Cu(II) and Cu(I), was proposed. The catalytic activity of Cu(II) towards O₂ reduction was found to decrease: free Cu(II) > Cu(II)-Aβ_{1-16} > Cu(II)-Aβ_{1-42} > Cu(II)-Aβ oligomer/fibril mixture > Cu(II)-Aβ fibrils [56].

The Aβ peptides interaction with Cu(II) in the presence of epigallocatechin-3-gallate (EGCG), based on the decrease of both oxidation peaks, of the Aβ peptide’s Tyr^{10} amino acid residue and of the EGCG, has been investigated [57]. A significant decrease in the SW voltammetric signal was observed after 2 h incubation, which indicated the chelation of Cu(II) ions by EGCG. Combining the voltammetric results with those obtained using spectrophotometry and transmission electron microscopy, it was found that Cu(II) ions interact with Tyr^{10} amino acid residue and this interaction is affected by the surrounding His residues [57].

The Aβ_{1-42}, Aβ_{25-35} and Aβ_{1-16} and mutant Aβ peptides interaction with Fe(III) [58] and Cu(II) [59], by CV and DP voltammetry, at BBDE and AFM, at mica surface, have been investigated. The AFM results showed that the Fe(III) ions induced the Fe(III)-Aβ_{1-42} peptide complexes fibrilization, the Fe(III)-Aβ_{1-16} peptide aggregation morphology was spherical and nonfibrillar [58] and the presence of Cu(II) disabled the Aβ peptides fibril formation [59]. The voltammetric results showed that Met^{35} amino acid residue was involved in the redox chemistry as a reducing agent. The Fe(III)-Aβ peptides complexes showed weaker binding ability when Tyr^{10} amino acid residue was substituted, evidencing that Fe(III) primarily binds to the Aβ peptide via the Tyr^{10} amino acid residue. These results were in contrast to Zn(II)- or Cu(II)-induced aggregation, where His residues acted as the primary metal binding sites [58,59].

The Aβ_{1-16} peptide interaction with the Fe(III) and Fe(II) complex with nitritriacetic acid (NTA), has been investigated [60]. The redox potential of the Fe(III)-NTA-Aβ_{1-16} peptide complex was negatively shifted when compared to the redox potential of free Fe(III)/Fe(II). Despite the large potential modulation, the redox potential of the Fe(III)-NTA-Aβ_{1-16} peptide complex was still sufficiently high for a range of redox reactions with cellular species to occur. The Fe(II)-NTA-Aβ_{1-16} peptide complex electrogenerated from the Fe(III)-NTA-Aβ_{1-16} peptide complex was also found to catalyse the reduction of O₂ to produce H₂O₂. In the presence of a biological reductant (antioxidant), Fe redox cycling could disrupt the redox balance within the cellular milieu. As a consequence, not only are ROS continuously produced, but O₂ and biological reductants can also be depleted. A cascade of biological processes could therefore, be affected. In
addition, the strong binding affinity of Aβ1-16 peptide towards Fe(III) and Fe(II) indicated that the Aβ1-16 peptide could compete for Fe against other Fe-containing proteins [60].

5. ELECTROCHEMICAL BIOSENSORS FOR Aβ PEPTIDES DETECTION

The brain pathological changes in AD take place years before the clinical manifestation of the disease, therefore, the identification and validation of biomarkers for diagnosing AD in early stages are increasingly important. The research on AD biomarkers focused primarily on the cerebrospinal fluid (CSF), since it is in direct contact with the brain extracellular space and reflects directly the biochemical changes that occur in the brain [61,62].

The Aβ1-42 peptide is considered a potential biomarker for the early AD detection and monitoring the disease progression [63]. The initial and predominating Aβ peptide deposited in diffuse plaques is the faster aggregating Aβ1-42 peptide [61]. Studies on cognitively healthy patients, at risk for AD, showed that the CSF’s concentrations of both Aβ1-42 peptide and protein tau phosphorylated at threonine 181 (pTau181) are altered. The Aβ1-42 peptides decreased concentration on the AD patients’ CSF was hypothesized to be caused by the deposition of Aβ1-42 peptides in plaques, leading to lower levels of Aβ1-42 peptides diffusing to the CSF. The specific detection of Aβ1-42 peptide biomarker in biological fluids, using electrochemical biosensors, has been investigated [64-69].

An electrochemical biosensor for the detection of Aβ1-42 peptide in a microfluidic chip architecture has been developed [64]. The electrochemical biosensor was based on the CV detection of the K₃[Fe(CN)₆]/K₄[Fe(CN)₆] label on colloidal gold nanoparticles (AuNPs), bound through 1,6-hexanediol linkers onto a gold working electrode, the gold electrode modification with AuNPs providing a suitable environment for stable Aβ1-42 peptides immobilization, keeping its bioactivity in a microreactor environment. The Aβ1-42 peptide aggregation, dependent on the peptide concentration, led to the electrode blocking and to the sensor output current decrease [64].

A disposable electrochemical immunosensor for the Aβ1-42 peptide detection, developed on SPCE nanostructured with AuNPs generated in situ, has been described [65]. The electrochemical immunosensing strategy consisted in a competitive immunoassay: biotin-Aβ1-42 peptide immobilized on the electrode surface and the Aβ1-42 peptide analyte competed for the anti-Aβ1-42 antibody. The electrochemical detection was carried out using an alkaline phosphatase labelled anti-rabbit IgG antibody. The electroanalytical current by CV, was based on the anodic stripping of enzymatically generated silver [65].

An impedimetric nanostructured biosensor, which used AuNPs uniformly deposited on gold thin film sputtered onto an anodic aluminum oxide layer with a nanohemisphere array, has been developed [66]. Using an Aβ1-42 antibody, the Aβ1-42 peptides aggregation and concentration have been evaluated by electrochemical impedance spectroscopy (EIS).

An electrochemical strategy for the Aβ peptides detection, based on gold nanoparticles (AuNPs) modified with Aβ1-16-heme (Aβ1-16-heme-AuNPs) anchored onto a gold electrode via a monoclonal antibody (mAb) specific to the common N-terminus of Aβ peptides, has been described [67]. The anchored Aβ1-16-heme-AuNPs showed a strong electrocatalytic O₂ reduction. Pre-incubation of the mAb-covered electrode with native Aβ peptides decreased the amount of Aβ1-16-heme-AuNPs immobilized onto the electrode, resulting in the decrease of the reduction current of O₂ to H₂O₂. The competitive assay was sensitive and selective to Aβ peptides, and the voltammetric response was proportional to the Aβ peptides concentration. The sensor was tested in artificial CSF containing Aβ1-40, Aβ1-42 and Aβ1-16 peptides [67].

A label-free electrochemical immunosensor for detection of the Aβ1-42 peptides, used a gold electrode modified with mercaptopropionic acid self-assembled monolayer, electrodeposited AuNPs and mAb DE2B4 as recognition element [68]. The biosensor was characterised by scanning electron microscopy, SW voltammetry and EIS.

Plastic antibodies mimic the recognition properties of biological materials and are traditionally obtained by molecularly-imprinted polymer technology [69]. A plastic antibody for the Aβ1-42 peptide was assembled by electropolymerizing the sugar monomer α-cyclodextrin (α-Cd) in the presence of the Aβ1-42 peptide, followed by the removal of the entrapped Aβ1-42 peptide by acid [69]. The plastic antibody was used for the development of an electrochemical biosensor for the Aβ1-42 peptide detection, consisting of a screen-printed gold electrode coated with a polyaniline (PANI) film and modified by the plastic antibody (MCd/PANI/Au-SPE) [69]. The ability of the
MCd/PANI/Au-SPE film to rebind Aβ1-42 peptides was followed by EIS and SW voltammetry, and compared to a control film without plastic antibody (NCd/PANI/Au-SPE). The overall method was evaluated, optimized and applied to the quantitative detection of Aβ1-42 peptides in fetal bovine serum samples.

Although the Aβ1-42 peptide concentration in CSF is important for AD diagnosis and prognosis, the Aβ1-42 peptide levels may differ by gender and age, thus the detection of the only Aβ1-42 peptide might be unable to discriminate between healthy patients, AD and/or other types of dementia [70]. Another methodology, consisting of monitoring the Aβ1-40/Aβ1-42 peptides ratio (or Aβ ratio) has been established as a reliable test to diagnose AD through human clinical trials [71].

A sensitive and selective electrochemical method for the detection of both the Aβ1-42 peptides and the total Aβ peptide content, using p-aminophenol (p-AP) redox cycling on an antibody-modified gold electrode, has been reported [70]. The conjugates achieved between streptavidin-conjugated alkaline phosphatase (SA-ALP) and biotinylated Aβ peptides, were captured by the antibody-modified electrodes, which induced the decrease of native Aβ peptides with the increase of native Aβ peptides concentration. To demonstrate the viability of the method for analysis of Aβ1-42 and total Aβ peptides in real samples, artificial CSF, containing Aβ1-40, Aβ1-42 and Aβ1-16 peptides, was tested [70].

Using a gelsolin-based electrochemical assay for Aβ peptide detection, the Aβ1-40/Aβ1-42 peptides ratio was detected in a rat model of AD [72]. A sandwich-type electrochemical biosensor has been prepared, using multi-walled CNTs and AuNPs, as substrates for the immobilisation of gelsolin, a secretory protein present in the CSF and plasma, onto SPCEs. The Aβ1-40/Aβ1-42 peptides were specifically recognized by the gelsolin tethered to the electrochemical biosensor in the presence of Th labels, followed by the binding of gelsolin-Au-Th bioconjugates. The Th reduction was used for the quantitative analysis of Aβ peptides. The soluble Aβ peptides level, in the CSF and in various brain regions, was found to be lower in rats with AD than in healthy rats [72].

A multiplexed, implantable electrochemical immunosensor to detect Aβ peptide isoforms using triple barrel carbon fiber microelectrodes as a sensor platform has been developed [71]. Antibodies, which act as biorecognition elements immobilised on the electrode surface, selectively captured and bound the Aβ1-40 and Aβ1-42 peptides, detecting them by measuring the Tyr oxidation peak current [71].

A label-free impedimetric immunosensor for the detection of Aβ1-42 and Aβ1-40 peptides, based on disposable electrochemical printed (DEP) chip, has been developed [73]. Three impedimetric immunosensor architectures and the bare electrode were compared, showing that AuNPs self-assembled monolayer surface modification increased the sensor sensitivity. A further modification using protein G, which helps orientate antibodies to an optimum position for the interaction with antigen, lowered the limit of detection. The presence of bovine serum albumin, one of the most abundant proteins in biological fluids, did not interfere with the sensitivity of the sensor [73].

In CSF, there is also a heterogeneous mixture of different length Aβ peptides, including Aβ1-42, Aβ1-40, Aβ1-37, Aβ1-38, Aβ1-39 and Aβ16-23 [61]. In AD patients, along with a decrease in Aβ1-42, increased CSF levels of both Aβ1-40 and Aβ1-38 were found, the diagnostic prospect of these Aβ variants being under current investigation.

A label-free electrochemical immunosensor for the detection of Aβ1-40 peptides, fabricated via immobilizing specific Aβ antibodies onto an interdigitated gold electrode (IDE-Au), modified by a dithiobis(succinimidyl propionate self-assembled monolayer), has been developed [74]. The Aβ1-40 peptide antibody has also been detected, using a layer-by-layer electrochemical immunosensor, containing silk fibroin and Aβ1-40 peptides onto SPCE [75].

The advanced glycation end products (AGEs) are compounds derived from the non-enzymatic protein glycation and oxidation break-down products [76]. Specific AGE compounds, due to their interactions with the receptor for AGEs (RAGE), have been involved in the progression of chronic diseases, such as diabetes, chronic inflammation, AD and cancer [76,77]. Two electrochemical biosensors for Aβ16-23 peptide detection, that used thiol-derivatives of nitrotriacetate acid-Cu(II) complex, for covalent and oriented immobilization of His-tagged domains of RAGE on the surface of gold electrodes, were developed [78]. The electrochemical biosensor active layers were the redox
active nitrilotriacetic acid$_{single}$-Cu(II)-His$_{6}^{x}$-receptor for AGE V domain and the redox active nitrilotriacetic acid$_{double}$-Cu(II)-His$_{6}^{x}$-receptor for AGE V domain complexes. The preparation procedure consisted of: (1) formation of a mixed layer composed of N-acetylcysteamine and thiol derivatives of nitrilotriacetic acid, (2) complexation of Cu(II) on the N-acetylcysteamine-nitrilotriacetic acid layer and (3) immobilization of natural and mutant Hys-tagged V domains, on the surface of a gold electrode modified with a N-acetylcysteamine-nitrilotriacetic acid. The electrochemical biosensors were used to study the interactions between the Hys-tagged V domains of RAGE and the Aβ$_{16-23}$ and Aβ$_{1-40}$ oligomers, each step being controlled by CV, SW voltammetry and AFM. The electrochemical parameters of each biosensor, incorporating single or double nitrilotriacetic acid molecules, were investigated [78].

An electrochemical biosensor that used the Hys-tagged V and VC1 domains of RAGE immobilized on a gold electrode surface, as analytically active molecules for the determination of Aβ$_{16-23}$ and Aβ$_{1-40}$ peptides, has been developed [79]. The immobilization of His$_{6}$-RAGE domains mixed layer on the gold electrode surface consisted of: (1) the deposition of N-acetylcysteamine (NAC) and thiol derivative of pentetic acid (DPTA), (2) Cu(II) complexation by DPTA and (3) His$_{6}$-RAGE domains oriented immobilization via coordination bonds between DPTA-Cu(II) complex and imidazole nitrogen atoms of a Hys tag. Each modification step was controlled by CV, SW voltammetry and AFM [79].

The Aβ oligomers have been also proposed as AD biomarkers, and levels of Aβ oligomers in the blood have been found to correlate with the CSF load.

A label-free impedimetric biosensor for the specific detection of Aβ oligomers, which is the primary neurotoxic species in AD, has been developed [80]. The biosensor biorecognition element consisted on a fragment of the cellular prion protein (PrPC, residues 95-110), a highly expressed synaptic protein which mediates the neuronal binding and toxicity of Aβ oligomers. During the layer-by-layer sensor construction, biotinylated PrPC(95-110) interacted specifically with the Aβ oligomers in solution, losing the capability to bind AgNPs and to induce the formation of an AgNPs-based network on the electrode. Consequently, the electrochemical current decrease was proportional to the Aβ oligomers concentration [83].

CONCLUSION

The recent research concerning the electrochemical characterisation of the Aβ peptides redox behaviour, Aβ peptides fibrilization process, Aβ peptides interaction with metal ions, as well as the use of electrochemical biosensors for the detection of Aβ peptides biomarkers for AD, was revised.

The full length Aβ$_{1-40}$ and Aβ$_{1-42}$ peptides redox analytes contain five electroactive amino acid residues: one tyrosine (Tyr$^{10}$), three histidine (His$^{6}$, His$^{13}$ and His$^{14}$) and one methionine (Met$^{35}$), the most remarkable contribution being from Tyr$^{10}$ amino acid residue, due to its high oxidation current. The Aβ peptides show two oxidation peaks, the first from the Tyr amino acid residue and the second from the three His and one Met amino acid residues. During the Aβ peptides
fibrillation process, in the absence/presence of metal ions, changes in the Tyr, His and Met amino acid residues oxidation currents occur. The difference in the Aβ peptides electrochemical behaviour explains the sensing strategy of the direct, label-free electrochemical detection of Aβ peptides conformational changes that take place during fibrillation and interactions with metal ions. The electrochemical biosensors research focused on the fast and specific detection of Aβ peptides biomarkers for AD in CSF and other biological fluids.

The use of electrochemical methods, such as CV, DP, SW voltammetry and EIS, for the investigation of Aβ peptides is continuously increasing, and become an important complementary tool in biochemical studies related with Aβ peptides aggregation, and clarification of AD neurodegeneration mechanisms.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Financial support from Fundação para a Ciência e Tecnologia (FCT), Grant SFRH/BPD/92726/2013 (A.-M. Chiorcea-Paquim), projects UID/EMS/00285/2013 (co-financed by the European Community Fund FEDER), FEDER funds through the program COMPETE - Programa Operacional Factores de Competitividade, and Innovec’EAU (SOE1/P1/F0173), are gratefully acknowledged.

REFERENCES

[25] Suprun, E.V.; Khmeleva, S.A.; Radko, S.P.; Kozin, S.A.; Archakov, A.I.; Shumyanseteva, V.V. Direct electrochemical oxidation of amyloid-β peptides via tyrosine; Histidine, and


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E.E. Electroanalysis of the interaction between (-)-epigallocatechin-3-gallate (EGCG) and amyloid-β in the presence of copper. Metallomics, 2013, 5(3), 259-264.


