Alzheimer's disease amyloid beta peptides in vitro electrochemical oxidation

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A B S T R A C T

The oxidative behaviour of the human amyloid beta (Aβ1–40 and Aβ1–42) peptides and a group of similar peptides: control inverse (Aβ1–40, and Aβ1–42), mutants (Aβ1–40Phe10 and Aβ1–40Ne54), cat Aβ1–40Rat, and fragments (Aβ1–20, Aβ1–15, Aβ1–10, Aβ1–12, 28, and Aβ1–42), in solution or adsorbed, at a glassy carbon electrode, by cyclic and differential pulse voltammetry, were investigated and compared. Structurally the Aβ1–40 and Aβ1–42 sequences contain five electroactive amino acid residues, one tyrosine (Tyr10), three histidines (His6, His13 and His14) and one methionine (Met35). The Aβ peptide 3D structure influenced the exposure of the redox residues to the electrode surface and their oxidation peak currents. Depending on the amino acid sequence length and content, the Aβ peptides gave one or two oxidation peaks. The first electron transfer reaction corresponded to the tyrosine amino acid residue oxidation, and the second to both histidines and methionine amino acid residues. The highest contribution to the second oxidation peak current was from His13, followed by His14 and His6 residues, and Met35 residue had the lowest contribution. The Aβ peptides electron transfer depended on peptide hydrophobicity and 3D structure, the redox residues position in the sequence, the redox residues close to N-termini giving the highest oxidation peak currents. © 2016 Elsevier B.V. All rights reserved.

1. Introduction

Alzheimer's disease (AD) was first described in 1906 by the German psychiatrist and neuropathologist Alois Alzheimer [1–3]. It is a common form of dementia and is an incurable, progressive, and terminal neurodegenerative disease [3–5]. The AD brain histopathology is characterized by the presence of intracellular neurofibrillary tangles of the hyper-phosphorylated Tau protein and by extracellular amyloid plaques containing a mixture of amyloid beta (Aβ) peptides of ~40 amino acids, having different N- and C-termini, generated from the larger amyloid protein precursor (APP) after proteolytic cleavage by β- and γ-secretases [4,6,7]. From a physiologic point of view, the predominant constituent species of amyloid plaques are Aβ1–40 (~90%) and Aβ1–42 (~10%) [3,8,9]. However, in a small amount, the shortest Aβ peptide sequences were also found as constituents of senile plaques [3,6,9,10], and presented a high analytic interest for understanding the aggregation, reactivity, and toxicity of human Aβ1–40 and Aβ1–42 peptides [11].

The toxicity mechanism, the specific toxic species, as well as the in vivo aggregation processes of human Aβ peptides, are still unclear. What is known till now is that in vitro, depending on the experimental conditions, the fibril formation begins with the Aβ peptide monomer predominantly unstructured (random coil), or with α-helical structure, and small reactive transient soluble species, formed during the aggregation process, are responsible for the toxicity [3,12–19], Schematic 1.

The first report about purification and identification of the Aβ monomer was in 1984 [10]. The 'amyloid hypothesis' was enunciated in the early 1990s by Selkoe, Hardy and Higgins, which postulated that the neurodegeneration in AD is caused by the deposition of Aβ peptide plaques in the brain tissue [6,20]. Nowadays, neither the plaques nor the Aβ monomeric units are considered the main pathobiologic agents in AD. What appeared to be primarily responsible to the neurodegeneration are transient heterogeneous small soluble oligomers (five or six Aβ peptide monomer units) and protofibrils (structures observed during formation of mature Aβ fibrils) [3,4,8,12–18,21]. As a consequence, obtaining structural information on the Aβ peptide monomers and their mechanistic pathways to form amyloid oligomers and fibrils has been and remains a major challenge.

Although the nature and the formation of the amyloid oligomers and fibrils has been intensively and successfully probed using a number of experimental techniques, the Aβ peptide monomer characterization remains difficult due to its tendency to aggregate [3]. Only a limited number of NMR studies have been reported on fragments of the Aβ peptide monomer and on full length peptides in which the Met35 residue was oxidized [22–26].

The investigation of Aβ peptides started 30–40 years ago, and the major goal was the aggregation process research. Generally, the working protocol had two parts: first the lyophilized peptides were dissolved in organic solvents (HFIP, ammonia, etc.), in order to be in the monomeric state, and then aggregation was promoted under high-salt conditions (usually chloride).
The 3D structures of the three Aβ peptides in Fig. 1 showed an α-helical structure for Aβ1–28, a mixed random coil and α-helical structure for Aβ1–40, and an unstructured random coil structure for Aβ1–42. Fig. 1. The position of the electroactive amino acids, tyrosine (Tyr), histidine (His) and methionine (Met), being very important to investigate the Aβ peptide sequences electrochemical oxidation.

In the human Aβ1–40 and Aβ1–42 peptide sequences, the Tyr10 and His13 are more accessible to the electrode surface enabling their easier oxidation, whereas His6, His14 and Met35 are less accessible and more difficult to be oxidized, Fig. 1.

The aim of the present study was the qualitative electroanalytical investigation of the oxidative behaviour of specifically selected twelve, different small and long, amyloid beta sequences, including the Aβ1–40 and Aβ1–42 human sequences, with the main goal to identify the side chain electroactive amino acid residue of human amyloid monomers, before the aggregation process, that may be involved in the Aβ electron transfer biochemical oxidation reaction. In order to limit the salt out effect induced by ionic species, defined by Hofmeister series, and minimize the aggregation rate, all experiments were performed immediately after preparation of the Aβ peptide solutions, in a free chloride media, in 0.1 M phosphate buffer supporting electrolyte, at the physiological pH 7.4, and the redox behaviour of twelve different Aβ peptide sequences, was investigated.

To better understand the human Aβ peptide oxidative process, twelve different amyloid structures, including relevant fragment sequences, also found in small amount as constituents of senile plaques, were investigated. The electron transfer properties of twelve different Aβ peptide sequences, human Aβ1–40 and Aβ1–42, inverse Aβ40–1 and Aβ42–1, fragments Aβ1–28, Aβ1–16, Aβ10–20, Aβ12–28, and Aβ17–42, mutants Aβ1–40Phe10 and Aβ1–40Nle35Aβ, and Aβ1–40Rat, Table 1, in solution and adsorbed, at the GCE surface, using voltammetric methods, were investigated. The inverse sequences contained the same amino acids as human Aβ peptide sequences, but in inverse order, are not affected by the aggregation process, and were used as control sequences.

Since the Aβ peptide participates in redox reactions in the extra- and intra-cellular medium, the qualitative electroanalytical study has the potential to provide valuable insights into the biological redox reactions of the human Aβ peptide sequences, resulting in a better understanding of the physicochemical properties of the different Aβ monomer structures and their aggregation processes that may occur in vivo, at different rates, and by different mechanistic pathways.

2. Experimental

2.1. Materials and reagents

The synthetic human Aβ1–40 and Aβ1–42, control inverse Aβ40–1 and Aβ42–1, short fragments Aβ1–28, Aβ1–16, Aβ10–20, Aβ12–28, and Aβ17–42, mutants Aβ1–40Phe10 and Aβ1–40Nle35Aβ, and Aβ1–40Rat...
peptide sequences, Table 1, were bought lyophilized from Sigma–Aldrich, Spain, and Bachem, Switzerland, and were used without further purification. Analytical-grade reagents and purified water from a Millipore Milli-Q system (Certificate ISO 9001 Quality systems standards) were used.

Prior to all experiments, the Aβ peptide solutions were prepared according to the following procedure. The lyophilized powder was firstly dissolved 1 mg/mL in 1,1,1,3,3-hexafluoro-2-propanol (HFIP) in order to monomerize pre-existing aggregates. After that, the HFIP was evaporated under a vacuum pump, leaving a film which was after re-dissolved in 0.1 M phosphate buffer supporting electrolyte, at physiological pH 7.4. The suspension was further monomerized by sonication for 1 min, followed by dilution to the desired concentration. The Aβ peptide monomers prepared solutions were transparent without evident aggregation. In all experiments the Aβ peptide solutions were used immediately after preparation.

2.2. Voltammetric parameters and electrochemical cells

Voltammetric experiments were carried out using an IVIUM potentiostat in combination with iviumSoft program version 2.219 (IVIUM Technologies, Eindhoven, The Netherlands). Measurements were carried out using a glassy carbon (GCE) working (d = 1 mm) (Edaq, Europe), a Pt wire counter and an Ag/AgCl (3 M KCl) reference electrodes, in a one-compartment 2 mL electrochemical cell. The experimental conditions were: for cyclic voltammetry (CV) scan rates 5 mV s\(^{-1}\) and 30 mV s\(^{-1}\), and for differential pulse (DP) voltammetry pulse amplitude 50 mV, pulse width 100 ms, and scan rate 5 mV s\(^{-1}\).

The GCE was polished using diamond spray (particle size 1 mm) before each experiment. After polishing, the electrode was rinsed thoroughly with Milli-Q water. After this mechanical treatment, the GCE was placed in 0.1 M phosphate buffer pH 7.4 supporting electrolyte and various DP voltammograms were recorded until a steady state baseline voltammogram was obtained. This procedure ensured very reproducible experimental results.

The immobilization of an Aβ peptide film on the GCE surface was carried out by covering the clean GCE surface with 5 μL from the 50 μM Aβ peptide solution and allowed to adsorb spontaneously for 10 min. The electrode was then rinsed several times with Milli-Q water. The GCE with the immobilized Aβ peptide film was then placed in the electrochemical cell, containing only the 0.1 M phosphate buffer pH 7.4 supporting electrolyte, and the DP voltammograms were recorded.

For all voltammetric experiments only freshly prepared Aβ peptide solutions were used in order to avoid the effect of aggregation process. All the voltammograms presented were background-subtracted and baseline-corrected using the iviumSoft program tools. This mathematical treatment was used for a better and clearer identification of the peaks. The voltammetric results were replicated three times and the R.S.D. obtained for each peptide was lower than 8%.

3. Results

The electron transfer properties of twelve different Aβ peptide sequences, human Aβ\(_{1-20}\), Aβ\(_{1-40}\) and Aβ\(_{1-42}\), control inverse Aβ\(_{40-1}\) and Aβ\(_{42-1}\), fragments Aβ\(_{1-28}\), Aβ\(_{1-16}\), Aβ\(_{10-20}\), Aβ\(_{12-28}\), and Aβ\(_{17-42}\), mutants Aβ\(_{1-40}\)Phe\(^{10}\) and Aβ\(_{1-40}\)Nle\(^{35}\)Aβ, and Aβ\(_{1-140}\)Rat, Table 1, directly in solution or after the peptide adsorption on the GCE surface, were investigated.

The oxidation of Aβ peptides either adsorbed or in solution, using voltammetric techniques, took place only at the electrode surface, and only tyrosine, histidines and methionine electroactive amino acid residues, were detected.

The amino acid type, number and sequence, dictated the 3D morphological conformations and the intramolecular interactions between side chain residues and the GCE surface, causing different voltammetric responses. There was no correlation between the twelve Aβ peptide sequences molecular weight and electroactive amino acid in the sequence, or Aβ peptide sequences molecular weight and hydrophobicity, Table 2.

Only the position of the electroactive amino acids in the Aβ peptide sequences 3D structures and their hydrophobicity, Fig.1, will determine their electroactive amino acid adsorption accessibility to the hydrophobic GCE surface, enabling the voltammetric detection.

3.1. Small Aβ peptide sequences

In order to understand the redox behaviour of the Aβ peptides of ~40 amino acids, AD plaques constituents, relevant shortest Aβ peptide sequences, existing in small amount as constituents of senile plaques, having different N- and C-termini, Aβ\(_{1-28}\), Aβ\(_{1-16}\), Aβ\(_{10-20}\), Aβ\(_{12-28}\), and Aβ\(_{17-42}\), Table 1, were investigated and compared.

This small Aβ peptide sequences were chosen in order to clarify the oxidation potentials and the influence of the electroactive amino acid residues position in the longer Aβ peptide sequences.
Table 1
The human Aβ_{40} and Aβ_{42} control inverse Aβ_{40-1} and Aβ_{42-1}, fragments Aβ_{1-28}, Aβ_{1-16}, Aβ_{10-28}, and Aβ_{12-28}, mutants Aβ_{1-40}Phe^{10} and Aβ_{1-40}Nle^{35}, and Aβ_{1-40}Rat peptides amino acid sequences.

|        | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 |
|--------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Aβ_{1-16} | Asp | Ala | Phe | Arg | His | Asp | Ser | Gly | Phe | Val | His | His | Gln | Lys |
| Aβ_{1-28} | Asp | Ala | Phe | Arg | His | Asp | Ser | Gly | Phe | Val | His | His | Leu | Val | Phe | Phe | Ala | Glu | Asp | Val | Gly | Ser | Asn | Lys |
| Aβ_{10-20} | Asp | Ala | Phe | Arg | His | Asp | Ser | Gly | Phe | Val | His | His | Leu | Val | Phe | Phe | Ala | Glu | Asp | Val | Gly | Ser | Asn | Lys |
| Aβ_{12-28} | Asp | Ala | Phe | Arg | His | Asp | Ser | Gly | Phe | Val | His | His | Leu | Val | Phe | Phe | Ala | Glu | Asp | Val | Gly | Ser | Asn | Lys |
| Aβ_{17-42} | Asp | Ala | Phe | Arg | His | Asp | Ser | Gly | Phe | Val | His | His | Leu | Val | Phe | Phe | Ala | Glu | Asp | Val | Gly | Ser | Asn | Lys |
| Aβ_{1-40} | Asp | Ala | Phe | Arg | His | Asp | Ser | Gly | Phe | Val | His | His | Leu | Val | Phe | Phe | Ala | Glu | Asp | Val | Gly | Ser | Asn | Lys |
| Aβ_{1-40Phe^{10}} | Asp | Ala | Phe | Arg | His | Asp | Ser | Gly | Phe | Val | His | His | Leu | Val | Phe | Phe | Ala | Glu | Asp | Val | Gly | Ser | Asn | Lys |
| Aβ_{1-40Nle^{35}} | Asp | Ala | Phe | Arg | His | Asp | Ser | Gly | Phe | Val | His | His | Leu | Val | Phe | Phe | Ala | Glu | Asp | Val | Gly | Ser | Asn | Lys |
| Aβ_{1-40Rat} | Asp | Ala | Phe | Arg | His | Asp | Ser | Gly | Phe | Val | His | His | Leu | Val | Phe | Phe | Ala | Glu | Asp | Val | Gly | Ser | Asn | Lys |

- Hydrophobic region;  Xaa - Electroactive amino acid residue
Table 2
Electroactive amino acids, molecular weight and hydrophobicity of Aβ peptides.

<table>
<thead>
<tr>
<th>Aβ peptide</th>
<th>Molecular weight</th>
<th>His⁶</th>
<th>Tyr¹⁰</th>
<th>His¹³</th>
<th>His¹⁴</th>
<th>Met³⁵</th>
<th>Hydrophobicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ₁₋₁₅</td>
<td>1955.01</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>18.75</td>
</tr>
<tr>
<td>Aβ₁₋₂₈</td>
<td>3262.46</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>32.14</td>
</tr>
<tr>
<td>Aβ₁₀₋₂₀</td>
<td>1446.65</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>45.54</td>
</tr>
<tr>
<td>Aβ₁₂₋₂₈</td>
<td>1955.18</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>41.18</td>
</tr>
<tr>
<td>Aβ₁₇₋₄₂</td>
<td>2577.05</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>61.54</td>
</tr>
<tr>
<td>Aβ₁₋₄₀</td>
<td>4329.80</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>42.50</td>
</tr>
<tr>
<td>Aβ₁₋₄₂</td>
<td>4514.04</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>45.24</td>
</tr>
<tr>
<td>Aβ₄₀₋₁</td>
<td>4329.80</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>42.50</td>
</tr>
<tr>
<td>Aβ₄₂₋₁</td>
<td>4514.04</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>45.24</td>
</tr>
<tr>
<td>Aβ₄₂₋₂₈</td>
<td>4312.80</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>45</td>
</tr>
<tr>
<td>Aβ₄₀₋₄₅Phe¹⁰</td>
<td>4233.72</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>45.54</td>
</tr>
<tr>
<td>Aβ₄₀₋₄₅Rat</td>
<td>4233.72</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>45</td>
</tr>
<tr>
<td>Aβ₄₀₋₄₅Nle³⁵</td>
<td>4313.80</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>-42.50</td>
</tr>
</tbody>
</table>

* Predicted information about Aβ₄₀₋₄₅Nle³⁵ hydrophobicity.

The Aβ₁₋₁₅ and Aβ₁₋₂₈ sequences presented four electroactive amino acid residues: His⁶, Tyr¹⁰, His¹³ and His¹⁴, all in the middle of the chain. However, in the Aβ₁₋₁₅ sequence the His¹³ and His¹⁴ are two amino acid residues away from the C-termini.

The short Aβ₁₀₋₂₀ sequence presented three electroactive amino acid residues: Tyr¹⁰ at the N-termini, followed by two histidines, His¹³ and His¹⁴, in the middle of the chain.

The Aβ₁₂₋₂₈ sequence presented only electroactive His amino acid residues, His¹³ and His¹⁴, and Met³⁵ amino acid residues in the Aβ₁ peptide chain.

3.2. Longer Aβ peptide sequences

The human Aβ₁₋₄₀ and Aβ₁₋₄₂ peptide sequences, and the control inverse Aβ₄₀₋₁ and Aβ₄₂₋₁ peptide sequences, both presented the five electroactive amino acid residues, whereas in the mutant sequences, Aβ₄₀₋₄₅Phe¹⁰ peptide lacked the Tyr¹⁰, and Aβ₄₀₋₄₅Nle³⁵ peptide lacked Met³⁵.

The Aβ₄₀₋₄₅Rat peptide sequence deserves special attention, as rats are not affected by AD [11,45,46], and presented three amino acids substitutions: Gly⁵ instead of Arg⁵, Phe¹⁰ instead of Tyr¹⁰, and Arg¹³ instead of His¹³, when compared with the human Aβ₁₋₄₀ peptide sequence.

Analyzing the hydrophobicity of the small Aβ₁₋₁₅ and longer Aβ₁₋₄₀ and Aβ₁₋₄₂ peptides monomers, Fig. 1 and Table 1, were in the hydrophobic scale blue is the least, and red the most hydrophobic region, two high hydrophobic domains: Lys¹⁰₋₁₆ and Val²₄₋₄₂, can be observed. Besides these regions, the Aβ₁ peptide monomers hydrophobic and hydrophilic amino acid residues were mixed, with no strong evidence of hydrophobicity.
3.3. The effect of concentration, adsorption time and incubation time

As the Aβ40 peptide is present in 90% of the amyloid plaques, this Aβ peptide is a very relevant analyte, and the influence of Aβ1–40 peptide concentration and adsorption time on the voltammetric behaviour of the Aβ40 peptide adsorbed film on the GCE, in 0.1 M phosphate buffer pH 7.4, was investigated.

Varying the Aβ40 peptide adsorption time and concentration it was found that the optimal condition for the preparation of the Aβ40 peptide adsorbed film on GCE required 50 μM Aβ40 peptide solution and 10 min adsorption time.

Assuming that an experiment requested maximum 15 min (10 min adsorption and 5 min DP voltammetric scan) an aggregation test was made, in which the peptide was incubated in solution for 30 min. The DP voltammograms were recorded before and after Aβ40 peptide incubation, Fig. 3. The DP voltammetric behaviour of Aβ1–40, in solution or adsorbed at the GCE surface, remained the same, meaning that at these experimental conditions the Aβ4 peptide morphology did not change. The aggregation process of Aβ1–42 is faster than that of Aβ1–40 but not spontaneous and not linear [34]. The aggregation process is, for both peptides, sigmoidal with a very long lag phase time, almost half of the total aggregation time.

3.4. Cyclic voltammetry

Cyclic voltammetry (CV) is one of the most versatile techniques for the study of electroactive species. It is an electroanalytical tool to monitor and detect the reversibility of many electrochemical processes taking place at the electrode surface and can be used to study redox processes in biochemistry and macromolecular chemistry [47].

CVs of longer Aβ peptide sequences, Aβ1–40 and Aβ1–42, and the control inverse Aβ peptide sequences, Aβ40–1 and Aβ42–2, in 0.1 M phosphate buffer pH 7.4, in solution and adsorbed at the GCE surface, were carried out, Fig. 4.

The CVs for adsorbed control inverse Aβ40–1 peptide, at the electrode surface, showed two anodic irreversible peaks, (Fig. 4). The first oxidation peak, at $E_p = +0.65$ V, corresponded to the Tyr10 residue, and the second broader oxidation peak, at $E_p = +1.00$ V, corresponded to the contribution of the His6, His13, His14 and Met35 residues oxidation in the Aβ peptide chain [43].

For an irreversible oxidation reaction, the CV peak current, in amperes is $i_p = \frac{2.99 \times 10^2 (nF_\alpha)^{1/2} A R_\alpha D_{\alpha} \Gamma^{1/2}}{R_\alpha}$, where $n$ is the number of electrons transferred, $i_p$ is the number of electrons in the rate-determining step, $A$ is electroactive area in cm$^2$, $D_{\alpha}$ is the diffusion coefficient in cm$^2$ s$^{-1}$, $R_\alpha$ is the concentration in mol cm$^{-3}$ and $\Gamma$ is the scan rate in V s$^{-1}$ [47].

Considering that the control inverse Aβ40–1 peptide sequence contained only one Tyr10 residue, and that the Tyr10 adsorbed amino acid residue was oxidized during the first scan, the surface concentration, $\Gamma$, in mol cm$^{-2}$ of the adsorbed control inverse Aβ40–1 peptide was calculated as $\Gamma = Q (nF A)^{-1} = 3.82 \times 10^{-12}$ mol cm$^{-2}$, where $Q$ represented the charge in coulombs, $n$ is the number of electrons transferred, and $A$ is the GCE geometrical area in cm$^2$. This surface concentration was specific for an Aβ1 monolayer film [35].

CVs at 30 mV s$^{-1}$, at GCE, in 0.1 M phosphate buffer pH 7.4, in solutions containing 50 μM of Aβ1–40, Aβ1–42, Aβ40–1, and Aβ42–2, were recorded, Fig. 4. For all Aβ peptide sequences investigated two irreversible oxidation peaks, at the same potential as those of adsorbed Aβ40–1 peptide, with smaller oxidation peak currents, occurred. These differences in peak current are explained by the Aβ peptide sequences different 3D structures, hydrophobicity, and diffusion coefficient, that will determine their electroactive amino acid adsorption accessibility to the hydrophobic GCE surface, enabling the voltammetric detection.

3.5. Differential pulse voltammetry

DP voltammetry is a differential method, the response is similar to the first derivative of a conventional voltammogram [47,48], the scan

<table>
<thead>
<tr>
<th>Peptide</th>
<th>1st peak</th>
<th>E (V)</th>
<th>SD</th>
<th>RSD (%)</th>
<th>$I$ (nA)</th>
<th>SD</th>
<th>RSD (%)</th>
<th>2nd peak</th>
<th>E (V)</th>
<th>SD</th>
<th>RSD (%)</th>
<th>$I$ (nA)</th>
<th>SD</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ1–10</td>
<td>0.64</td>
<td>0.005</td>
<td>0.8</td>
<td>0.643</td>
<td>0.048</td>
<td>7.5</td>
<td></td>
<td>1.01</td>
<td>0</td>
<td>0</td>
<td>1.302</td>
<td>0.098</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>Aβ1–28</td>
<td>0.64</td>
<td>0</td>
<td>0</td>
<td>2.118</td>
<td>0.178</td>
<td>8.4</td>
<td></td>
<td>1.00</td>
<td>0.005</td>
<td>0.5</td>
<td>2.986</td>
<td>0.233</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td>Aβ1–40</td>
<td>0.63</td>
<td>0.011</td>
<td>1.8</td>
<td>0.842</td>
<td>0.068</td>
<td>8.1</td>
<td></td>
<td>1.01</td>
<td>0</td>
<td>0</td>
<td>1.456</td>
<td>0.041</td>
<td>2.8</td>
<td></td>
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<tr>
<td>Aβ1–42</td>
<td>0.62</td>
<td>0.005</td>
<td>0.9</td>
<td>2.131</td>
<td>0.135</td>
<td>6.3</td>
<td></td>
<td>1.01</td>
<td>0.005</td>
<td>0.5</td>
<td>3.431</td>
<td>0.269</td>
<td>7.8</td>
<td></td>
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<tr>
<td>Aβ10–20</td>
<td>0.62</td>
<td>0.005</td>
<td>0.9</td>
<td>2.131</td>
<td>0.135</td>
<td>6.3</td>
<td></td>
<td>1.01</td>
<td>0.005</td>
<td>0.5</td>
<td>3.431</td>
<td>0.269</td>
<td>7.8</td>
<td></td>
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<tr>
<td>Aβ12–28</td>
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<td>0.005</td>
<td>0.9</td>
<td>1.661</td>
<td>0.028</td>
<td>1.7</td>
<td></td>
<td>1.00</td>
<td>0.005</td>
<td>0.5</td>
<td>0.99</td>
<td>0.005</td>
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<tr>
<td>Aβ17–42</td>
<td>0.60</td>
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<td>1.529</td>
<td>0.073</td>
<td>4.7</td>
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The qualitative electroanalytical behaviour, by DP voltammetry, of different \( \text{A}\beta \) peptide sequences, in solution or adsorbed at the GCE surface, in 0.1 M phosphate buffer pH 7.4, was investigated. For all DP voltammograms of \( \text{A}\beta \) peptides adsorbed at the GCE surface, Table 3, or \( \text{A}\beta \) peptides in solutions, Table 4, the oxidation peak potentials and currents, and standard and relative standard deviation, were determined.

rate cannot be higher than 10 mV s\(^{-1}\), and all theoretical aspects were considered when the DP voltammetric parameters were chosen [48].

3.5.1. \( \text{A}\beta \) peptides adsorbed onto the GCE

The twelve \( \text{A}\beta \) peptides were adsorbed onto the GCE for 10 min, forming a very reproducible monolayer film, and have been evaluated by DP voltammetry.

3.5.1.1. Adsorbed small \( \text{A}\beta \) peptide sequences. The DP voltammograms for adsorbed \( \text{A}\beta_{1-28}, \text{A}\beta_{1-16}, \text{A}\beta_{10-20} \) peptide sequences, in 0.1 M phosphate buffer pH 7.4, showed two oxidation peaks. The less positive oxidation peak corresponding to Tyr\(^{10} \) amino acid residue, at \( E_p = +0.65 \) V. The more positive oxidation peak potential corresponding to the contribution in the peptide chain from histidine amino acid residues, at \( E_p = +1.00 \) V. The \( \text{A}\beta_{1-28} \) and \( \text{A}\beta_{1-16} \) peptides containing...
three, His\textsuperscript{6}, His\textsuperscript{13} and His\textsuperscript{14}, and the A\textsubscript{β\textsuperscript{10–20}} peptide containing two, His\textsuperscript{13} and His\textsuperscript{14}, Fig. 5.

The adsorbed A\textsubscript{β\textsuperscript{12–28}} peptide presented only His\textsuperscript{13} and His\textsuperscript{14} electroactive amino acid residues in the peptide chain, and only one oxidation peak, at \( E_p = +1.00 \, \text{V} \), occurred, Fig. 5. This indicates that the His amino acid residues in the A\textsubscript{β\textsuperscript{12–28}} peptide chain are oxidized at a slightly lower potential than free His amino acid.

The A\textsubscript{β\textsuperscript{17–42}} peptide is the only peptide investigated that has only one Met\textsuperscript{35} electroactive amino acid residue in the peptide chain. The Met\textsuperscript{35} oxidation, at \( E_p = +1.00 \, \text{V} \), Fig. 5, is at a slightly lower potential than free Met amino acid, and at the same potential as His amino acid residues in the A\textsubscript{β\textsuperscript{12–28}} peptide sequence.

Although there are very few reports about Met oxidation in peptides and proteins [49], the hydrophobic residues of A\textsubscript{β\textsuperscript{17–42}} peptide enabled a strong adsorption, with the Met\textsuperscript{35} residue in closer contact with the GCE surface, facilitating the electron transfer, in agreement with the lower oxidation potential for Met\textsuperscript{35}, in adsorbed A\textsubscript{β\textsuperscript{17–42}} peptide obtained by DP voltammetry, Fig. 5.

The oxidation peak potentials for His\textsuperscript{13} and His\textsuperscript{14} in adsorbed A\textsubscript{β\textsuperscript{12–28}} peptide, and Met\textsuperscript{35} in adsorbed A\textsubscript{β\textsuperscript{17–42}} peptide, were slightly lower than for free His and Met amino acids. This is explained taking into account the 3D structures of A\textsubscript{β\textsuperscript{12–28}} and A\textsubscript{β\textsuperscript{17–42}} peptides, and the hydrophobic interactions between the hydrophobic side chain residues and the electrode surface, which allowed a strong A\beta peptide adsorption, with the His\textsuperscript{13} and His\textsuperscript{14} or Met\textsuperscript{35} amino acid residues in closer contact with the GCE surface, facilitating the electron transfer. For the other A\beta peptides sequences investigated, the Met\textsuperscript{35} oxidation always occurred at a potential close to the His residues oxidation potential, making it impossible to separate the two oxidation peaks, that will always be superimposed.

The A\textsubscript{β\textsuperscript{1–40}} peptide sequence contained three histidine residues, His\textsuperscript{6}, His\textsuperscript{13} and His\textsuperscript{14}, while the A\textsubscript{β\textsuperscript{12–28}} and A\textsubscript{β\textsuperscript{10–20}} peptide sequences contained only two residues, His\textsuperscript{13} and His\textsuperscript{14}. The hydrophobicity of A\textsubscript{β\textsuperscript{12–28}}, A\textsubscript{β\textsuperscript{1–40}} and A\textsubscript{β\textsuperscript{10–20}} peptides lies between 45.45% and 31.14%, the relationship being A\textsubscript{β\textsuperscript{10–20}} > A\textsubscript{β\textsuperscript{12–28}} > A\textsubscript{β\textsuperscript{1–40}}. Table 2.
Comparing the oxidation peak currents from the DP voltammograms of adsorbed Aβ1–10 of Aβ1–28 peptide sequences with those of adsorbed Aβ1–16 and Aβ1–28 peptide sequences, Fig. 5, it was observed that, in Aβ1–10 and Aβ1–28 peptide sequences, the His13 and His14 oxidation peak currents are higher than the oxidation peak currents with added His6, in Aβ1–16 and Aβ1–28 peptide sequences.

Also the Aβ1–16 peptide has the lowest hydrophobicity, 18.75%, although containing the same electroactive residues as the Aβ1–28 peptide, Table 2, and the Aβ1–16 peptide oxidation peak current was much lower than that of the Aβ1–28 peptide, Fig. 5.

The Aβ3 peptides hydrophobic properties are very important for their adsorption, and were taken into consideration. The position of electroactive amino acid residues relative to hydrophobic/hydrophilic residues in the Aβ3 peptide sequences, affected the electron transfer at the GCE surface, and the electroactive residues close to the electrode surface presented fast electron transfer, Schematic 2.

The accessibility of the electroactive amino acid residues to the GCE surface will be greater, for an adsorbed Aββ peptide, when the electroactive amino acid residues are contained into a hydrophobic region, and the electrochemical signal, the oxidation peak current, will be higher compared with the oxidation peak current of the electroactive amino acid residue contained in a less accessible to the GCE hydrophilic region.

3.5.1.2. Adsorbed longer Aβ peptide sequences. The adsorbed Aβ1–42 peptide presented two oxidation peaks, Fig. 6, with the same potentials and currents of adsorbed Aβ1–16 peptide, Fig. 5.

Similar behaviour was also observed for adsorbed Aβ1–40 peptide and the adsorbed control inverse Aβ40–1 and Aβ32–1 peptides. All DP voltammograms recorded for these sequences presented two oxidation peaks at the same potentials and with the same currents, except for adsorbed Aβ40–1 peptide which presented higher oxidation peak currents, Fig. 6.

The DP voltammogram for adsorbed mutant Aβ1–40Phe10 peptide sequence showed only the oxidation of histidines (His6, His13 and His14) and Met35 residues, at Ep = + 1.00 V, Fig. 7. However, the adsorbed Aβ1–40Phe10 peptide oxidation peak current was very high compared with adsorbed Aβ1–40 peptide, suggesting that the substitution of Tyr10 in Aβ1–40 gave rise to a Aβ1–40Phe10 peptide 3D structure that adsorbed to the GCE surface in a more favourable orientation for the three histidines (His6, His13 and His14) and Met35 residues oxidation to occur, Fig. 7.

The DP voltammogram for the same conditions obtained for adsorbed Aβ1–40Ram peptide, Fig. 7, showed very small histidines (His6 and His14) and Met35 amino acid residues oxidation peak current.

These results indicated that even minor changes on the peptide primary structure, such as one amino acid substitution, lead to a different secondary structure and its adsorption influenced the amino acid residue accessibility to the GCE surface and the amino acid residue oxidation current.

This was confirmed for adsorbed Aβ1–40 peptide sequences where adsorption favoured the oxidation of more exposed His13 and His14 residues, with His6 being less accessible to the electrode surface, Fig. 1.

3.5.2. Aβ peptides in solution

DP voltammetry of 50 μM Aβ peptides of different 3D structures in solution, in 0.1 M phosphate buffer pH 7.4, was investigated. As already observed for adsorbed Aβ peptides, depending on the Aβ peptides primary structure, the results showed one or two oxidation peaks corresponding to the Tyr10, His6, His13, His14, and Met35 amino acid residues, Figs. 8 and 9.

3.5.2.1. Small Aβ peptide sequences in solution. DP voltammetry in solution showed that the highest oxidation peak currents were obtained for the Aβ10–20 peptide, Fig. 8. The Aβ10–20 Peptide Tyr10 oxidation peak current, Ip = 15 nA, at Ep = + 0.65 V, was almost three times higher than the Tyr10 oxidation peak current in Aβ1–16 or Aβ1–28 peptides, and this was due to the fact that Tyr10 in Aβ10–20 peptide is the N-termini amino acid, easier to be oxidized because, being the first amino acid residue in the Aβ peptide sequence, is more accessible to the GCE surface. Additionally, the hydrophobicity of Aβ10–20 Peptide of 45.54% facilitates the N-termini Tyr10 oxidation, when compared with the middle chain Tyr10 in Aβ1–16 peptide, which has the lowest hydrophobicity, 18.75%.

The Aβ12–28 peptide only oxidation peak was due to the His residues, since it did not contain Tyr10, or Met35, and the current was similar to that in Aβ1–28 and Aβ1–16 peptides oxidation but lower than the current of Aβ10–20 peptide, Fig. 8.

The Aβ10–20 peptide has only 11 amino acid residues and is more likely to be a random coil rather than an α-helix structure, as happened with Aβ1–28 peptide, Fig. 1, and this explained the Tyr10 and His oxidation peak currents different in Aβ10–20 peptide compared to other small Aβ peptide sequences, Fig. 8.

3.5.2.2. Longer Aβ peptide sequences in solution. The DP voltammograms in human Aβ1–40, Aβ1–42, and mutant Aβ1–40Ne35 and Aβ1–42Phe10 peptide sequences, Fig. 9, showed one or two oxidation peaks with small differences between the oxidation peak currents. However, the Tyr10 oxidation peak current for all longer Aβ peptide sequences was always higher than the His and Met amino acid residue oxidation peak current.

4. Discussion

Even at the very beginning of protein sequence and structure discovery, it was very clear that the position and properties of the amino acids in the peptide sequence were crucial to understand many biological processes.

The side chain amino acid properties, and the specific arrangement pattern of the amino acid residues, compatible with the formation of β-strands (i, i + 2) or compatible with the formation of α-helices (i, i + 4), strongly affected the Aβ peptides 3D structure. The α-helix formation is governed by amino acid intrinsic helix-forming propensity, and is characterized by the formation of hydrogen bonds between the backbone N—H group of i + 4 residue, and the C=O group of the amino acid i, four residues earlier.

The differences between oxidation currents of different Aβ peptides 3D structures are due to the fact that each Aβ peptide represents a different molecule, with different properties. The i-1 and i + 1 neighbours of tyrosine residue of Aβ1–40 are Gly and Glu, while for the control inverse Aβ40–1, are Glu and Gly. Regarding Aβ1–42 and control inverse Aβ32–1, the residues Ile-Ala, are at the carboxyl terminal, and Ala-Ile, are at the amino terminal, completely changing these Aβ3 peptides properties.

The investigation of substituted Aβ peptide sequences was essential to understand how the Aβ peptide primer structure influenced the secondary structure and favoured the electroactive amino acid residue oxidation.

The highest oxidation peak currents occurred for peptide Aβ10–20 (Tyr10 near N-termini, His13, His14), and for hydrophobic peptide sequences Aβ12–28 (His13 near N-termini, His14), and Aβ1–28 (His6, Tyr10, His13, His14), which contained the electroactive amino acid residues in the middle of the chain.

Small oxidation peak currents were obtained for the smaller peptide Aβ1–16 (His6, Tyr10, His13 and His14 near C-termini), which was the less hydrophobic, and for peptide Aβ17–42 (Met35), where Met35 was the only electroactive amino acid.

The experiments in solutions containing different small Aβ peptide sequences confirmed the Tyr10 high oxidation peak current, when Tyr10 amino acid residue was situated close to the N-termini, Aβ10–20 peptide, and that the Tyr10 oxidation peak current was always greater than the His and Met35 oxidation peak current.
On the adsorbed human Aβ1–40 and Aβ1–42 peptide sequences, the Tyr10 and His13 were more accessible to the GCE surface enabling their easier oxidation, whereas His6, His14 and Met35 were less accessible and more difficult to be oxidized, Fig. 1.

In human Aβ peptides, the Tyr10 amino acid has a helical propensity of 0.53 kcal/mol, and Tyr10 is four amino acid residues equidistant, on the left from His6 and on the right from His13, and the His amino acid has an helical propensity of 0.61 kcal/mol. This means that for the α-helix secondary structure the Tyr10 phenol side chain will be in close proximity with the His6 and His14 imidazole moieties.

In mutant Aβ1–40Ne35 and Aβ1–40Phe10 peptides and Aβ1–40Rat peptide sequences, where one or more non-electroactive amino acids substituted the electroactive amino acids of the human Aβ1–40 peptide sequence, was observed that even the substitution of a single non-electroactive amino acid induced changes on the oxidative behaviour to the human Aβ1–40 peptide.

The adsorbed mutant Aβ1–40Phe10 peptide showed a huge increase of His and Met β1 oxidation peak current. Similarly, for the adsorbed mutant Aβ1–40Ne35 peptide the Tyr10 oxidation peak current also increased. Unlike the phenylalanine non-electroactive side chain amino acid in mutant Aβ1–40Phe10 peptide, tyrosine in the human Aβ1–40 peptide contains a reactive hydroxyl group, which will be involved in interactions with non-carbon atoms, e.g. –OH –N. This interaction explains the great increase of His oxidation peak current in Aβ1–40Phe10 peptide, compared with human Aβ1–40 peptide.

The substitutions in the Aβ1–40Rat peptide sequence, Gly5 instead of Arg5, Phe10 instead of Tyr10, and Arg13 instead of His13, affect the electroactive His susceptibility for oxidation, by the neighbours His6 near Gly5, and His13 near Arg13, when compared with the human Aβ1–40 peptide sequence. The Aβ1–42Rat peptide has only three His, His14, and Met35 electroactive amino acid residues compared with human Aβ1–42 with five, His5, Tyr10, His13, His14, and Met35 electroactive amino acid residues but the second oxidation peak current was similar. The fact that rats are not affected by AD [11,45,46], and the electroactive amino acid residues but the second oxidation peak current was similar. The fact that rats are not affected by AD, and electroactive amino acids Tyr10 and His13 are missing in the Aβ1–42Rat peptide sequence, support the conclusion that Tyr10 and His13 amino acids may have a stronger contribution to the human Aβ1 peptides oxidation and may be significantly involved in Alzheimer’s disease neurodegeneration.

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