



# Electrochemical oxidation of amyloid-beta peptide isoforms on carbon screen printed electrodes



Elena V. Suprun<sup>a,\*</sup>, Sergey P. Radko<sup>a,b</sup>, Svetlana A. Khmeleva<sup>a,b</sup>, Vladimir A. Mitkevich<sup>b</sup>, Alexander I. Archakov<sup>a</sup>, Alexander A. Makarov<sup>b</sup>, Victoria V. Shumyantseva<sup>a</sup>

<sup>a</sup> Institute of Biomedical Chemistry, Pogodinskaya Street, 10/8, Moscow 119121, Russia

<sup>b</sup> Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilov Street, 32, Moscow 119991, Russia

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## ABSTRACT

The direct electrochemical oxidation of synthetic analogs of the 42 amino acid long amyloid-beta peptide (A $\beta$ 42) and its isoforms with familial mutations (D7H or H6R) and the naturally occurring modification (Ser-8 phosphorylation) has been investigated on carbon screen printed electrodes in the phosphate buffer (pH 7.2) by cyclic and square wave voltammetry. For all peptides tested, the oxidation current exhibited a peak ( $I_p$ ) at the potential maximum ( $E_{max}$ ) of about 0.6 V, a small peak or wave at 0.9–1.2 V and a large wave at 1.2–1.5 V (vs. Ag/AgCl), respectively corresponding to the electrochemical oxidation of Tyr-10, His (His-6, His-13, and His-14), and Met-35 residues. To confirm the oxidation of Met-35 in A $\beta$ 42 peptides, the short peptide A $\beta$ 16 lacking the Met was employed. Each A $\beta$ 42 isoform under study has displayed a specific oxidation voltammogram within the region of 0–1.5 V. The Tyr-10 based electrochemical analysis allowed to discriminate A $\beta$ 42 from some of its isoforms, based on the  $I_p$  and the  $E_{max}$  values. It was shown that the oxidation currents of Tyr, His, and Met residues decrease upon the aggregation of A $\beta$ 42 and its isoforms and can therefore be utilized to monitor the peptides' aggregation.

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## 1. Introduction

The aggregation of amyloid- $\beta$  (A $\beta$ ), a 39–42 amino acid long peptide, is commonly implicated in the Alzheimer's disease onset and development. It has been the focus of attention of many investigators for decades [1–3]. Various biophysical and biochemical techniques are currently employed to study A $\beta$  aggregation in vitro in the search for a cure for that deadly disease [4]. Among them, the direct electrochemistry appears to present itself as a rapid and cost-effective approach [4–6].

The basics of proteins' and peptides' electroactivity via reduction or oxidation of some amino acid residues are well established presently [7–13]. Consequently, the electrochemical oxidation of A $\beta$  is determined by three types of amino acid residues: Tyr-10, His (His-6, -13, and -14), and Met-35. The oxidation of A $\beta$ 40 and A $\beta$ 42 via Tyr-10 at 0.6–0.7 V (vs. Ag/AgCl) was first shown on glassy carbon electrode (GCE) about a decade ago and utilized for monitoring the peptides' aggregation kinetics [14]. Later on, the Tyr-10 oxidation of A $\beta$ 40 and A $\beta$ 42 was employed to investigate effects of different organic compounds and divalent metal ions as well as variations in experimental conditions on

the peptides' aggregation [6,15–19]. The Tyr-10 electrooxidation has also been used to characterize some isoforms of A $\beta$ 16 (the truncated form of A $\beta$  representing its metal-binding domain) and their complexing with divalent metal ions [20–21], and to develop an electrochemical biosensor for A $\beta$  detection [22].

Earlier, we have shown for the first time that A $\beta$ 42 can be electrooxidized via His and Met-35 residues (additionally to the Tyr-10 oxidation) from a drop of sample placed on carbon screen printed electrode (SPE) [23]. Square wave voltammograms of A $\beta$ 42 peptide, registered in phosphate buffer (pH 7.2) within the potential range of 0–1.5 V, showed a peak at 0.6 V, a small peak at the potential of 1.05 V and a wave at about 1.0–1.5 V (vs. Ag/AgCl). Upon electrooxidation of free Tyr, His, and Met amino acids as well as A $\beta$ 16 peptides varying in the number of His and Tyr residues, the oxidation signals observed at 0.6 V, 1.05 V and 1.0–1.5 V were attributed to the electrooxidation of Tyr-10, three His and Met-35 residues, respectively [23]. The oxidation of Tyr, His, and Met residues was previously shown for some other peptides, using various types of carbon based electrodes [7–13,24]. Each type of carbon electrodes allows one to detect protein (peptide) with different sensitivity. To register an oxidation signal, a pre-treatment of electrode surface and a specific protocol of measurements are usually required [7–14,19,24]. Thus, the oxidation of methionine sulfoxide reductase A peptide via Tyr, Trp (with the potential maximum ( $E_{max}$ ) of

\* Corresponding author.

E-mail address: [lenasuprun@mail.ru](mailto:lenasuprun@mail.ru) (E.V. Suprun).

about 0.6 V (vs. Ag/AgCl)), and His ( $E_{\max}$  close to 0.9 V) residues was demonstrated at the neutral pH on a GCE by differential pulse voltammetry (DPV) [10]. The oxidation, by the alternating voltammetry, of angiotensin peptides (containing Tyr and His electroactive residues) on a basal plane pyrolytic graphite electrode has been reported by Palecek and co-workers [11]. In the tested peptides, Tyr residues produced the oxidation peak at the potential close to 0.7 V (vs. Ag/AgCl, pH 7.0). However, the oxidation of His residues produced no well-defined peak but just an increase of the oxidation current in the vicinity of 1 V in a proportion to the number of His residues in the peptide. Oxidation of Met residue was investigated under acidic conditions (pH 2.0) by the cyclic voltammetry on a boron-doped diamond electrode, using model dipeptides [24]. All Met-containing dipeptides tested were found to produce on voltammograms the large waves between potentials of 1.2 and 1.8 V or peaks with the  $E_{\max}$  of about 1.5 V (vs. SCE).

Recently, the electrooxidation of A $\beta$ 42, A $\beta$ 40, and rat A $\beta$ 40 peptides has been investigated by the DPV on a GCE in the phosphate buffer (pH 7.4) at potentials from 0.2 to 1.2 V (vs. Ag/AgCl) [19]. Two consecutive oxidation peaks have been found on the background-subtracted and baseline-corrected DPV voltammograms. Considering the first anodic signal at  $E_{\max} = 0.65$  V as corresponding to the Tyr-10 oxidation, the authors have attributed the second peak at  $E_{\max} \approx 1$  V to a sum of oxidation signals from Met-35 and three His residues [19]. However, the substitution of Met-35 residue in A $\beta$ 40 with the non-electroactive Nle-35 residue has failed to produce a significant decrease of the second oxidation peak [19]. In our opinion, it makes a contribution to this peak from Met-35 oxidation rather questionable. It is noteworthy that the amino acid substitution appears to be a straightforward approach to testing a role of a particular electroactive residue in the overall electrooxidation of A $\beta$  peptides. However, the results thus obtained should be treated with a caution since the present knowledge of how amino acid substitutions can alter the peptide's conformation and aggregation state is still limited. Clearly, differences in the conformation and the aggregation state may affect the accessibility of various electroactive groups to the oxidation on the electrode surface.

The present work aims to study the electrochemical behavior of A $\beta$ 42 and its isoforms with familial mutations [25,26] and the naturally occurring serine phosphorylation [27] in the potential range of 0 to 1.5 V on carbon SPE by the cyclic (CV) and square wave voltammetry (SWV). To elucidate the influence of these amino acid substitutions and the modification as well as the peptide's aggregation on oxidation signals of Tyr-10, three His, and Met-35, the values of peak currents ( $I_p$ ) and  $E_{\max}$  were estimated and compared. Besides, the oxidation of A $\beta$ 42 peptides via Met-35 at potentials of 1.2–1.5 V has been confirmed using A $\beta$ 16.

## 2. Experimental

### 2.1. Chemicals and peptides

A $\beta$ 16, A $\beta$ 42 and its isoforms were obtained as peptide lyophilisates (purity > 95%) from Biopeptide Co., LLC (USA). The peptides sequences are shown in Table 1. All chemicals were purchased from Sigma–Aldrich (USA) and were of an analytical grade or higher. The Milli-Q quality water was used to prepare all solutions.

Before use, A $\beta$ 42 and its isoforms were pretreated with hexafluoroisopropanol as described elsewhere [6] to minimize the amount of pre-existing A $\beta$  aggregates. The pretreated peptides were dissolved in  $1 \times 10^{-2}$  M NaOH at the peptide concentration of  $5 \times 10^{-4}$  M. The use of NaOH instead of dimethyl sulfoxide to dissolve peptides [23] has improved the reproducibility of electrochemical measurements. The peptide solutions in  $1 \times 10^{-2}$  M NaOH were diluted twice with  $1 \times 10^{-2}$  M Na-phosphate buffer, pH 6.4 (to the final pH 7.2). The lyophilized A $\beta$ 16 was dissolved in water [23]. The A $\beta$ 42 and A $\beta$ 16 peptide solutions were adjusted with the Na-phosphate buffer (pH 7.2) supplemented with NaCl so as to provide the peptide

solutions with concentration of either  $5 \times 10^{-5}$  M or  $1 \times 10^{-4}$  M in  $1 \times 10^{-2}$  M phosphate buffer (pH 7.2) containing  $5 \times 10^{-2}$  M NaCl. The peptide solutions were kept on ice until further use.

### 2.2. Apparatus and electrochemical measurements

CV and SWV measurements were performed using an Autolab electrochemical system PGSTAT-12 equipped with GPES software (Eco Chemie, Utrecht, The Netherlands). Carbon SPE and connector for SPE were made by Rusens Ltd. (Moscow, Russia, [www.rusens.com](http://www.rusens.com)). The electrochemical cell consisted of carbon working, carbon counter and silver reference electrodes. All potentials were referred to the silver screen printed pseudo-reference electrode (Ag/AgCl). The potential of pseudo-reference electrode was measured in phosphate buffer supplemented with  $\text{Cl}^-$  at concentration of  $5 \times 10^{-2}$  M. The diameter of the working electrode was 0.18 cm. The electrochemical experiments were carried out at room temperature ( $25 \pm 3$  °C). A 60- $\mu\text{L}$  aliquot of the tested solution was placed on the surface of SPE so as to cover all three electrodes. CV measurements were carried out in the range 0–1.5 V at the scan rate 5–100  $\text{mV s}^{-1}$ . The following parameters of SWV were used: equilibration time, 10 s; amplitude, 40 mV; step potential, 5 mV; square wave frequency, 25 Hz. The initial and end potentials were 0 and 1.5 V, respectively. Each SPE was utilized for a single measurement in order to avoid the blockage of the electrode surface by oxidation products of peptides. Each sample was tested with five independent measurements. The corresponding confidential intervals were calculated using the Student's *t*-distribution for the confidence level, *P*, being equal to 0.95 ( $t = 2.57$ ).

## 3. Results and discussion

Fig. 1A and 1B present SWV data on the oxidation of the A $\beta$ 42 and its isoforms in the potential range of 0–1.5 V. The voltammograms of all peptides tested exhibit three consecutive regions with specific alterations of the oxidation current: a peak at the  $E_{\max}$  of 0.6 V, a wave at 0.9–1.2 V, and a large wave in the region of 1.2–1.5 V. The peak and the small and large waves may be ascribed, respectively, to the oxidation of the Tyr-10, His (His-6, His-13 and/or His-14), and Met-35 residues based on the presently known electrochemical behavior for these amino acid residues [7–14,23,24]. The values of these oxidation signals varied for the peptides studied (Fig. 1A and 1B). Measurements had to be repeated at least five times to statistically distinguish some A $\beta$ 42 isoforms from each other. Contrary to Enache et al. [19], we suggest that oxidation of A $\beta$ 42 via Met-35 residue occurs at the potentials exceeding 1 V and differing from the  $E_{\max}$  value for the His residue oxidation [23]. Indeed, based on the results on the oxidation of Met-containing dipeptides [24] and free Met and His amino acids [23,28,29], one may expect that, depending on pH and electrode type, the measured current for Met oxidation should be several times higher than that for His residue. The SWV data on the oxidation of the A $\beta$ 42 and A $\beta$ 16 peptides (the latter contains Tyr-10 and three His residues but lacks Met-35), presented in Fig. 1C, support our suggestion. Compared to A $\beta$ 16, A $\beta$ 42 demonstrates a smaller peak of Tyr-10 oxidation at about 0.6 V, a “blurry” signal in the region typical for His oxidation, and a large wave at the potentials of 1.2–1.5 V, which is lacking on the A $\beta$ 16 voltammogram. It appears highly likely, therefore, that the large wave at 1.2–1.5 V is generated by Met-35 oxidation. It should be noted that the oxidation of  $\text{PO}_3^{2-}$  group also occurs at high potential values (around 1.4 V as shown for a free phosphorylated Tyr [30]) and may thus account for the elevated oxidation signal for pS8-A $\beta$ 42 isoform compared to A $\beta$ 42 in the potential range of 1.2–1.5 V (Fig. 1B). Moreover, all three electrooxidation signals observed for A $\beta$ 42 and its mutants have exhibited a steady decrease with time upon incubation of peptide solutions at room temperature, as exemplified in Fig. 1D with the D7H-A $\beta$ 42 peptide, most probably due to the peptides' aggregation [25]. CV analysis of the A $\beta$ 16, A $\beta$ 42 and its isoforms has shown that  $I_p$  values attributed to the oxidation of

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