Electrochemistry Communications 65 (2016) 53–56

Contents lists available at ScienceDirect

Electrochemistry Communications

journal homepage: www.elsevier.com/locate/elecom

Short communication

Direct electrochemical oxidation of amyloid-β peptides via tyrosine, histidine, and methionine residues

Elena V. Suprun [a,*], Svetlana A. Khmeleva [a], Sergey P. Radko [a,b], Sergey A. Kozin [b], Alexander I. Archakov [a], Victoria V. Shumyantseva [a]

[a] Institute of Biomedical Chemistry, Pogodinskaya Street, 10/8, Moscow, 119121, Russia
[b] Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilov Street, 32, Moscow, 119991, Russia

ARTICLE INFO

Article history:
Received 1 January 2016
Received in revised form 2 February 2016
Accepted 5 February 2016
Available online 15 February 2016

Keywords:
Amyloid-β peptide
Histidine
Methionine
Tyrosine
Oxidation

ABSTRACT

The direct electrochemical oxidation of synthetic peptides analogous to the amyloid-beta (Aβ) implicated in the pathogenesis of Alzheimer's disease has been demonstrated on carbon screen-printed electrodes in the phosphate buffer (pH 7.2) by using square wave voltammetry. The full-length peptide Aβ42 was found to produce two peaks at potentials of 0.6, 1.05, and a wave at about 1–1.5 V (*vs.* Ag/AgCl) assigned, respectively, to its Tyr, His, and Met residues. The correspondence between the oxidation signals and the appropriate residues was established based on the analysis of voltammograms obtained for the free Tyr, His, and Met amino acids; for the Aβ16 peptide (representing the Aβ metal-binding domain and lacking the Met residue); for Aβ16 mutants differing in the number of His residues; and for the rat Aβ16 lacking the Tyr residue. The oxidation signals were found to be proportional to the amino acid and peptide concentrations. The observed difference in the electrochemical behavior of Aβ peptides widens the application of direct electrochemistry to peptide differentiation and point mutation studies as well as to investigation of Aβ aggregation and complexing with metal ions.

1. Introduction

Aβ is a 39–42 amino acid long peptide found in the cerebrospinal fluid at nanomolar concentrations [1]. According to the amyloid cascade hypothesis, the transition of Aβ from the monomeric state to insoluble fibrillar aggregates, which constitute amyloid plaques, is a key event of Alzheimer's disease [2]. The synthetic peptide Aβ42 analogous to the full-length Aβ is widely employed in *in vitro* studies aimed at elucidating the molecular mechanisms of Alzheimer's disease, using various biophysical and biochemical techniques including electrochemical methods [3–5]. The synthetic peptide of 16 amino acids (Aβ16) represents the Aβ metal-binding domain and is commonly used to study Aβ complexing with metal ions [3,6–12]. It is noteworthy that Aβ16 corresponds to the C-truncated form of the native Aβ peptide [13].

To date, the application of direct electrochemistry to investigation of Aβ peptides was based on the oxidation of a Tyr-10 residue that occurs at the potential of 0.6–0.7 V (*vs.* Ag/AgCl) [14]. The Tyr-10 oxidation made it possible to monitor Aβ16, Aβ40, and Aβ42 aggregation [14–18]; study Aβ16 complexing with metal ions [19,20]; characterize Aβ16 mutants [20]; and develop Aβ40 (Aβ42) sensor [21]. In addition to Tyr-10 residue, Aβ contains three His (His-6, His-13, and His-14) and Met-35 residues—all potentially electroactive [5]. It should be noted that His residues are of interest for studying Aβ interactions with metal ions since they are directly involved in metal binding [22,23]. The Met-35 residue is located within the peptide's hydrophobic tail and, therefore, can serve as a useful electroactive "label" for monitoring Aβ aggregation. Upon peptide self-association, this residue is likely to be embedded in the aggregate interior, thus becoming completely inaccessible for oxidation. However, the oxidation of His and Met residues has not as yet been demonstrated for Aβ peptides. While the direct electrochemistry of proteins and peptides is commonly based on the oxidation of Tyr, Trp, and Cys residues [24–29], the protein (peptide) oxidation via Cys-Cys, His, and Met residues has been shown only in a few cases [26,30,31]. Therefore, a direct oxidation (along with Tyr) of His and Met residues of Aβ peptides, if revealed, would provide a promising tool for monitoring Aβ aggregation and for studying Aβ interactions with metal ions.

The aim of the present work was to elucidate whether a direct electrochemical oxidation of Aβ peptides via His and Met electroactive residues in addition to Tyr residue is actually possible. Oxidation voltammograms of Aβ peptides were registered within the potential range of 0 to 1.5 V by square wave voltammetry (SWV) on carbon screen-printed electrodes (SPE). For Aβ42, three electrooxidation signals were revealed (peaks at potentials of 0.6 V and 1.05 V, and a wave at about 1–1.5 V (*vs.* Ag/AgCl)) and attributed to Tyr, His, and Met residues, respectively. To confirm the signal origins, (a) free amino acids (Tyr, His, and Met), (b) the Aβ16 peptide and its mutants

* Corresponding author. Tel.: +7 499 246 58 20; fax: +7 499 245 08 57.
E-mail address: lenasuprun@mail.ru (E.V. Suprun).

http://dx.doi.org/10.1016/j.elecom.2016.02.004
1388-2481/

carrying the various numbers of His residues, and (c) the rat Aβ16 (rAβ16) peptide lacking the Tyr residue and representing the metal-binding domain of the rat Aβ were analyzed.

2. Experimental

2.1. Chemicals and peptides

Aβ peptides (purity >95%) were purchased from Biopeptide Co., LLC (USA). The sequences and designations of Aβ peptides used are presented in Table 1. Free L-amino acids (Tyr, His, and Met), hexafluoroisopropanol (HFIP), dimethyl sulfoxide (DMSO), and other chemicals used were from Sigma-Aldrich (USA). The chemicals were of an analytical grade or higher. The Milli-Q quality water was used to prepare all solutions.

The solution of Aβ42 peptide was prepared by closely following the protocol of Stine et al. [32]. The peptide was pretreated with HFIP, lyophilized, and dissolved in DMSO at the concentration 3.3×10^{-3} M. The peptide solution was diluted 10-fold with cold (4 °C) water and kept on ice. The peptide concentration was measured with the commercial BCA assay (Thermo Scientific, USA), employing bovine serum albumin (BSA) as a standard, and adjusted to 2×10^{-4} M with water. The protocol allows one to obtain Aβ42 peptide mostly in the monomeric state [32]. The lyophilized Aβ16 and its mutants were dissolved in water, and peptide concentrations were determined by UV absorption spectroscopy using the extinction coefficient of 1400 M^{-1} cm^{-1} at 275 nm (from Tyr-10 of Aβ) [3]. The lyophilized rAβ16 was dissolved in water and its concentration was determined with the BCA assay, using Aβ16 as a standard. The peptide concentrations were adjusted to 2×10^{-4} M with water and the peptide solutions were kept on ice until further use. All peptide solutions were diluted twice just prior to measurements with the appropriate buffer to provide 1×10^{-4} M solutions in 1×10^{-2} M phosphate buffer (pH 7.2) supplemented with 5×10^{-2} M NaCl.

2.2. Apparatus and electrochemical measurements

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). The planar three-electrode cell consisted of the carbon working, carbon counter and silver reference electrodes with the counter electrode encircling the working one. The dimensions of SPE were 2.7 × 0.9 cm with the working electrode's diameter of 0.18 cm. For both the working and the auxiliary electrodes, the carbon ink C2030519P4 from Gwent Group (UK) was used. All potentials were referred to the silver screen-printed pseudo-reference electrode (Ag/AgCl). The electrochemical experiments were carried out at room temperature (25 ± 3 °C). A 60-μL aliquot of the tested solution was placed on the SPE surface so as to cover all 3 electrodes. The following parameters of SWV were used: equilibration time, 10 s; amplitude, 40 mV; step potential, 5 mV; and square wave frequency, 25 Hz. The initial and end potentials were varied from 0 to 1.5 V (*vs.* Ag/AgCl): the scan range to measure Aβ Tyr residue oxidation was from 0.2 to 0.9 V; the scan ranges for Aβ oxidation via His and Met residues were 0.75–1.25 V and 0.75–1.5 V, respectively. Each SPE was utilized for a single measurement in order to avoid the blockage of the electrode surface by oxidation products of amino acids or peptides. Each sample was tested with three independent measurements. The relative standard deviation in all cases did not exceed 10%. The corresponding confidential intervals were calculated using the Student's *t*-distribution for the confidence level, *P*, being equal to 0.95 ($t = 4.3$).

3. Results and discussion

Fig. 1A presents SWV data on Aβ42 and Aβ16 oxidation obtained with the use of carbon SPE. In the Aβ42 voltammogram, three regions with the increased oxidation current were observed: the well-defined peak at 0.6 V, the small peak at 1.05 V, and a wave at about 1–1.5 V. As is known, Aβ42 has three types of potentially electroactive amino acid residues [5]: Tyr, His, and Met. While the electrooxidation of Aβ peptides of different lengths (Aβ42, Aβ40, Aβ16) via the Tyr-10 residue at potentials of 0.6–0.7 V (*vs.* Ag/AgCl) has already been demonstrated [14–21], the oxidation of other electroactive amino acid residues in Aβ is open to questions. In contrast to Aβ42, the Aβ16 exhibited only two electrooxidation signals at respective potential maxima (E_{max}) of about 0.6 V and 1.05 V (Fig. 1A). Since Aβ16 has no Met residue, one may suggest that the oxidation wave at about 1–1.5 V in case of the Aβ42 peptide is generated by Met-35. Indeed, the oxidation of free Met under the same experimental conditions was obtained within the same range of potentials (Fig. 1B), which correlates with results reported in [33]. The Aβ42 oxidation via Met-35 at potentials of about 1–1.5 V is also confirmed by the earlier reported data on BSA oxidation via Met residues [26]. The free His has exhibited a broad oxidative wave over the 1–1.5 V range (Fig. 1B). Based on the results reported for the oxidation of both the free His and the His residues in the methionine sulfoxide reductase A peptide [30], the peak at 1.05 V was attributed to Aβ42 and Aβ16 oxidation via His residues (Fig. 1A). Peak current (I_p) values obtained for Aβ42 and Aβ16 oxidation via His and Met residues are shown in Table 1. To confirm this assumption, the electrooxidation of several Aβ16 mutants differing in the number of His residues, along with rAβ16 electrooxidation was studied (Table 1). The data on I_p values at E_{max} of 1.05 V for all peptides tested, summarized in Table 1, agree well with the number of His residues in each peptide. As follows from Fig. 2A, I_p value at E_{max} of 1.05 V for rAβ16 (which has only two His residues, His-6 and His-14) was smaller than that obtained for Aβ16 with three His residues (His-6, His-13, and His-14). However, H6A-H13A-Aβ16, which still has a His-14, exhibited no signal at this potential. The His-14 residue may have quite different accessibilities for oxidation in Aβ peptides due to differences in their conformational states, as was previously demonstrated for the Aβ Tyr-10 residue [20].

Except for rAβ16 in which Tyr is lacking, Aβ peptides demonstrated well-defined oxidation peaks at E_{max} of 0.6 V (Fig. 2B), associated with Tyr-10 [14–21,24,25]. The observed differences in I_p values for Aβ peptides' oxidation via the single Tyr residue (Table 1) should be attributed

Table 1
The sequences of Aβ peptides tested and their oxidation signals via Tyr, His, and Met residues.*

Peptide name	Peptide sequence	I_p, μA			E_{max}, V		
		Tyr	His	Met	Tyr	His	Met
Aβ42	NH_2-DAEFRHDSGYEVHHQKLVFFAED VGSNKGAIIGLMVGGVVIA-OH	0.26 ± 0.06	0.02 ± 0.01	16 ± 6	0.61 ± 0.03	1.03 ± 0.05	1.4
Aβ16	Acetyl-DAEFRHDSGYEVHHQK-Amide	0.7 ± 0.1	0.08 ± 0.06	–	0.57 ± 0.02	1.03 ± 0.02	–
H6A-H13A-Aβ16	Acetyl-DAEFR**A**DSGYEV**A**HQK-Amide	0.4 ± 0.1	–	–	0.60 ± 0.03	–	–
D7H-Aβ16	Acetyl-DAEFRH**H**SGYEVHHQK-Amide	0.98 ± 0.08	0.12 ± 0.06	–	0.57 ± 0.01	1.04 ± 0.01	–
H6R-Aβ16	Acetyl-DAEFR**R**DSGYEVHHQK-Amide	0.8 ± 0.1	0.06 ± 0.01	–	0.57 ± 0.04	1.03 ± 0.01	–
rAβ16	Acetyl-DAEFGHDSGFEVRHQK-Amide	–	0.05 ± 0.03	–	–	1.05 ± 0.03	–

The amino acid substitutions are shown in bold.
* The data presented are the means of the detected I_p and E_{max} values with the corresponding confidential intervals ($P = 0.95$).