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Electrochemical Analysis of Amyloid-β Domain 1-16 Isoforms and Their Complexes with Zn(II) Ions

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ABSTRACT

Tyrosine based electrochemical analysis of various isoforms of the amyloid-β fragment 1-16 (Aβ16), representing the metal-binding domain of Alzheimer's human Aβ peptide with amino acid substitutions and post-translational modification (D7H, D7N, H6R, H6A-H13A, E11K, and pS8), was carried out by square wave voltammetry on carbon screen printed electrodes. Electrochemical analysis allowed for distinguishing: (i) some isoforms under study from the "normal variant" of the Aβ16; and (ii) the isoforms from one another. Effects of Zn(II) ions on Aβ16 isoforms' oxidation were studied within a wide range of Zn(II) ion concentrations in HEPES-buffers with the pH values of 5.5 to 9. Except for H6A-H13A-Aβ16, addition of Zn(II) ions significantly reduced the intensity of oxidation signals for Aβ16 and its isoforms and shifted the peaks to the more positive potentials. H6A-H13A-Aβ16 demonstrated distinctly different electrochemical behavior both in the absence and presence of Zn(II) ions. The observed effects were discussed in the light of known modes of the Zn(II) ions binding to Aβ16 and its isoforms. The proposed electrochemical assay based on the direct oxidation signal of a tyrosine residue emerges as a very promising tool for monitoring the conformational changes of Aβ peptides *in vitro* as well as for studying the effects of point mutations and amino acid modifications on the conformation of peptide-metal ion complexes.

1. Introduction

Alzheimer's disease (AD) is the most prevalent neurological disorder affecting tens of millions of people worldwide. According to the amyloid cascade hypothesis, the key event of the AD pathology is a transition of amyloid-β (Aβ, the 39–42 amino acid long peptide) from the monomeric into the aggregated state, which triggers a chain of adverse molecular events [1]. Details of the molecular mechanism underlying this process are still unclear. It is known that the Aβ aggregation can be mediated by Zn(II) ions which interact with Aβ through its metal-binding domain composed of N-terminal residues 1–16 [2]. Zn(II) ions are considered as important modulators of neurotransmission [3] and their concentration in a synaptic cleft during neuronal excitation can reach 3×10^{-4} mol dm^{-3} [2]. *In vivo* Aβ aggregation leads to formation of senile plaques in the cerebral cortex – i.e. the extracellular deposits composed mostly by Aβ and known to contain large amounts of Zn(II) ions [4,5].

In vitro formation of Aβ-Zn(II) complexes has been investigated using synthetic peptides analogous to Aβ and its metal binding domain, by means of different techniques including isothermal titration calorimetry, nuclear magnetic resonance (NMR), mass spectrometry, circular dichroism, X-ray absorption spectroscopy, and electrochemistry [6–12]. However, the synthetic analogues of the full-length Aβ isoforms, usually Aβ40 and Aβ42 (Aβ peptides of 40 and 42 amino acids long, respectively), undergo a rapid Zn (II)-induced aggregation [2] that, in most cases, masks the event of Aβ-Zn(II) complex formation. To overcome this problem, the more stable synthetic peptides of 16 amino acids long (Aβ16), representing both the metal-binding domain of the full-length Aβ and its C-truncated form found in the human cerebrospinal fluid [13], were commonly employed in studies on Aβ-Zn(II) interactions [9–11,14]. Moreover, some modifications of the Aβ16 termini such as acetylation/amidation [8,9] or PEGylation [11] were introduced in order to further enhance the resistance of Aβ16 peptides to the Zn(II)-induced aggregation. It should be noted that Aβ16 peptides which lack the hydrophobic tail (17–42)

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have no propensity to the shear-induced aggregation in contrast to the full-length Aβ peptides.

Electrochemical studies of protein/peptide interactions with metal ions can be based either on the redox activity of appropriate metal ion [15] or on the electroactivity of protein/peptide amino acid residues such as Cys, Trp, Tyr, His, Met, and Cys-Cys [16–19]. Direct electrochemistry of proteins is finding an increasing application in biomedical research [19] including the *in vitro* studies of protein aggregation implicated in neurodegenerative disorders [18]. It was shown also that the electrochemical signal arising upon protein oxidation is sensitive to protein denaturation and to changes in amino acid sequence. The signal was found to significantly increase upon protein unfolding by urea [17,20,21]. Importantly, the electrochemical approach allows for distinguishing between the wild-type and the mutant forms of acetylcholinesterase with single amino acid substitutions [22]. The authors of [22] have pointed out that changes in the acetylcholinesterase conformation related to these substitutions produce more profound effects on the oxidation signal of the mutants than the amino acid substitutions *per se*. The observed effects confirmed the suggestion that solely the amino acid residues localized on the protein surface can exhibit their electroactive properties [23].

The electroactive amino acid residues localized in the polypeptide chain of Aβ are those of His at positions 6, 13, and 14 (His-6, -13, and -14), Met at position 35 (Met-35), and Tyr at position 10 (Tyr-10). Except for Met-35, all of them are located in the metal-binding domain. However, oxidation of His and Met residues requires strong positive potentials exceeding 1 V (*vs.* Ag/AgCl) [24,25] and has not as yet been observed for Aβ. In contrast, Aβ is easily oxidized due to the Tyr residues at neutral pH on carbon electrodes at the potentials of 0.6–0.7 V (*vs.* Ag/AgCl) [26]. The Tyr based electrooxidation of Aβ40 and Aβ42 was employed for the electrochemical sensing of polysaccharide-peptide interactions and was suggested for development of Aβ sensor [27]. The electrochemical approach was successfully used for monitoring Aβ40 and Aβ42 aggregation kinetics [26] as well as for evaluation of effects of some chemical compounds and small peptides on the extent of Aβ aggregation [28,29]. It appears that Tyr-10 residues become progressively inaccessible for oxidation on the electrode surface upon Aβ aggregation since they are shielded by other amino acid residues of the aggregated peptides [26,28,29].

The direct electrochemistry has been utilized for studying the impact of Zn(II) ions on aggregation of the synthetic peptides Aβ42, Aβ40, and Aβ16 [30,31]. Zn(II) ions induced a dramatic decrease of the oxidation current in the case of Aβ42 and Aβ40 [30]. However, it was impossible to distinguish contributions to that decrease from the Zn(II)-binding event and the Aβ aggregation. Comparative study of the electrochemical oxidation of Aβ42 and Aβ16 peptides in the presence and absence of Zn(II) ions has revealed that Zn(II) ions, upon binding to Aβ16, can also induce the partial peptide aggregation [31], which hinders data interpretation in terms of the Aβ-Zn(II) complexes. Recently [32], we have studied Aβ16-Zn(II) interactions by the Tyr based electrooxidation of Aβ16, using the Aβ16 peptide with respectively acetylated and amidated N- and C-termini – the modifications making Aβ16 more resistant to the Zn(II)-induced aggregation and thus allowing us to study solely the event of the Aβ16-Zn(II) complexing. As was shown earlier, the electrochemical assay based on the direct oxidation signal of Tyr-10 residue suits well for monitoring the Zn(II)-induced conformational changes of Aβ16 *in vitro* [32]. Thus, Tyr-10 residue can act as an Aβ electroactive label sensitive to peptide concentration, conformational changes, and aggregation [30–32].

Here, we are reporting the electrochemical analysis of various isoforms of Aβ16, including those with the naturally occurring point mutations and post-translational modification [33–37]. Using the square wave voltammetry (SWV) and carbon screen-printed electrodes (SPE), the effects of amino acid substitutions and modification as well as the effects of Zn(II) ions on the Aβ16 peptide oxidation were investigated and considered in the light of literature data on Zn(II) ion interactions with Aβ16 isoforms. It appears that amino acid substitutions and modification influence the microenvironment of Tyr-10 residue, leading to variations in its oxidation signal. SWV on SPE can be a promising complement to the commonly adopted methods to monitor conformational changes in peptides, including metal-induced alterations.

2. Experimental

2.1. Chemicals and peptides

Aβ16 and its isoforms (purity > 98%) were purchased from Biopeptide Co., LLC (USA). The N- and C-termini of the peptides were protected with acetyl and amide, respectively. The sequences and designations of the peptides used are presented in Table 1. The lyophilized peptides were dissolved in deionized water and peptide concentrations were determined by UV absorption spectroscopy, using the extinction coefficient of 1400 mol^{-1} dm^3 cm^{-1} at 275 nm (from Tyr-10 of Aβ) [6]. The peptide concentrations were adjusted to 1×10^{-4} mol dm^{-3} with water to yield the stock solutions of peptides under study. These solutions were kept on ice before use. Stock solutions of Zn(II) ions were prepared in deionized water from $ZnCl_2$ (Sigma-Aldrich, USA). Ethylenediaminetetraacetic acid (EDTA) and other chemicals used were from Sigma-Aldrich. The chemicals were of an analytical grade or higher. For all solutions deionized water was used.

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 electrochemical cell consisted of carbon working, carbon counter and silver reference electrodes. All potentials were

Table 1
The sequences of Aβ16 and its isoforms. The amino acid substitutions and modifications are shown in bold. The references are provided for the naturally occurring mutations and modifications.

Peptide name	Peptide sequence	Reference
Aβ16	Acetyl-DAEFRHDSGYEVHHQK-Amide	–
pS8-Aβ16	Acetyl-DAEFRHD-**pS**-GYEVHHQK-Amide	[36]
D7N-Aβ16	Acetyl-DAEFRH**N**SGYEVHHQK-Amide	[33] (Tottory mutation)
D7H-Aβ16	Acetyl-DAEFRH**H**SGYEVHHQK-Amide	[34] (Taiwan mutation)
H6R-Aβ16	Acetyl-DAEFR**R**DSGYEVHHQK-Amide	[35] (English mutation)
H6A-H13A-Aβ16	Acetyl-DAEFR**A**DSGYEV**A**HQK-Amide	–
E11K-Aβ16	Acetyl-DAEFRHDSGY**K**VHHQK-Amide	–