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Label-free electrochemical detection of singlet oxygen protein damage

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ABSTRACT

Oxidative damage of proteins results in changes of their structures and functions. In this work, the singlet oxygen ($^1O_2$)-mediated oxidation of bovine serum albumin (BSA) and urease by blue-light photosensitization of the tris(2,2′-bipyridine)ruthenium(II) cation $[Ru(bpy)_3]^{2+}$ was studied by square wave voltammetry at glassy carbon electrode and by constant current chronopotentiometry at mercury electrode. Small changes in voltammetric oxidation Tyr and Trp peaks did not indicate significant changes in the BSA structure after photo-oxidation at carbon electrode. On the other hand chronopotentiometric peak H of BSA at HMDE increased during blue-light photosensitization, indicating that photo-oxidized BSA was more susceptible to the electric field-induced denaturation than non-oxidized native BSA. Similar results were obtained for urease, where enzymatic activity was also evaluated. The present results show the capability of label- and reagent-free electrochemical methods to detect oxidative changes in proteins. We believe that these methods will become important tools for detection of various protein damages.

1. Introduction

Proteins are one of the major targets for oxidative damage in the cell. Indirect non-radical oxidation of the protein via formation and subsequent reaction with singlet oxygen ($^1O_2$) is one of the major processes. $^1O_2$-mediated oxidation induces several biophysical and biochemical changes in proteins, such as an increase in susceptibility of the oxidized protein to proteolytic enzymes, alterations in mechanical properties, an increased extent or predisposition to unfolding, changes in conformation, an increase in hydrophobicity and changes in binding of co-factors and metal ions (reviewed in [1–3]). The extent of component damage does not need to correlate with the importance of damage. Thus a low level of damage to critical species may be of much greater significance than massive damage to a nonessential target. Oxidative damage in proteins has been studied by high-performance liquid chromatography, fluorescence spectroscopy, mass spectroscopy, 2D electrophoresis, electron paramagnetic resonance, Raman resonance [2–4], while the use of electrochemical methods for this purpose is still infrequent [5–7]. Wang et al. studied BSA damage by Fenton reaction mediated by the hydroxyl radical •OH, where the differential pulse voltammetric oxidation signal of electroactive indicator (2,2′-bipyridyl) cobalt(III) perchlorate decreased in correlation with BSA damage [7]. Indeed, •OH is one of the most reactive oxygen species that markedly non-selectively reacts with proteins, inducing protein damage such as protein fragmentation [3].

Electrochemistry of proteins boomed in the last decades, but it has focused on electroactivity of non-protein redox centers (such as metal ions) in relatively small number of conjugated proteins, being thus of little use in proteomics studying thousands of proteins [8,9]. A couple of studies of non-conjugated proteins were done using voltammetry at graphite electrodes [10–13] and impedance spectroscopy at metal electrodes [14]. We showed that chronopotentiometric stripping in combination with mercury-containing electrodes is suitable tool for label-free protein detection [13] including proteins important in biomedicine [12,15–18]. Nano and subnano-molar concentrations of proteins can be detected at lower stripping current intensities [13,19], while conformational changes in proteins [13,20,21] and also protein-DNA [22] interactions can be studied at higher current intensities.

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In this paper, we present new label- and reagent-free electrochemical methods suitable for detection of oxidative changes in proteins. We chose the non-radical oxidant $^1O_2$, which selectively reacts with electron-rich amino acid (aa) residues in proteins, such as Met, Cys, His, Tyr and Trp [3]. $^1O_2$ is more efficiently produced in tissues by UVA or visible light photosensitized reactions involving several endogenous and exogenous sensitizer molecules [23,24]. In our study, we followed BSA oxidative changes produced after blue-light photosensitization of tris(2,2'-bipyridine) ruthenium(II) cation ($Ru(bpy)_3^{2+}$) that generates $^1O_2$, by square wave voltammetry (SWV) at glassy carbon electrode (GCE) and by constant current chronopotentiometry (CPS) at mercury electrode. At carbon electrode no large $^1O_2$-mediated changes in the BSA voltammetric responses were detected. On the other hand CPS analysis at mercury electrode indicated that photo-oxidized surface-attached BSA and urease were destabilized and more susceptible to the electric field-induced denaturation.

2. Experimental

2.1. Materials

Bovine serum albumin, urease from jack bean, amino acids, polyamino acids and reagents of the highest available quality were received from Sigma-Aldrich. Solutions were prepared in triply-distilled water.

2.2. Electrochemical measurements

Voltammetric measurements were performed with μAutolab III analyzer (Eco Chemie, Utrecht, The Netherlands) in connection with VA-Stand 663 (Metrohm, Herisau, Switzerland). Three-electrode system were used containing Ag|AgCl|3 M KCl electrode as a reference and platinum wire as an auxiliary electrode. A hanging mercury drop electrode (HMDE, 0.4 mm^2, Metrohm, Switzerland) or a glassy carbon electrode (GCE, area 3.14 mm^2) were used as working electrodes. The cleaning procedure of the GCE included polishing with alumina 0.3 μm on soft lapping pads for 2 min followed by sonication by Fisherbrand FB 11020 in deionized water for 2 min. All measurements were performed at 26 °C open to air. CPS measurements at HMDE: stripping current, I_{str} −35 μA (if not stated otherwise). Square wave voltammetry (SWV) at GCE: amplitude 10 mV; step 10 mV, frequency 25 Hz. Voltammograms were baseline corrected with moving average employing the built-in GPES software.

2.2.1. Adsorptive (Ad, in situ) stripping at HMDE

BSA or aa's were adsorbed at accumulation potential, E_A + 0.1 V for accumulation time, t_A 60 s from 50 mM Na-phosphate pH 7 followed by chronopotentiogram recording. Stirring accompanied accumulation.

2.2.2. Adsorptive stripping at GCE

BSA or aa's were adsorbed at E_A + 0.1 V for t_A 300 s from 50 mM Na-phosphate pH 7 followed by SW voltammogram recording. Stirring accompanied accumulation.

2.2.3. Adsorptive Transfer (AdT, ex situ) at HMDE[25]

Proteins were adsorbed from 5 μL drop of 1 μM protein sample (if not stated otherwise) in 50 mM Na-phosphate pH 7 at open current potential for 2 min, followed by washing, transferring of protein modified HMDE to blank electrolyte and recording of chronopotentiogram.

2.2.4. Adsorptive Transfer (AdT, ex situ) at GCE

Proteins were adsorbed from 15 μL drop of 1 μM protein sample (if not stated otherwise) in 50 mM Na-phosphate pH 7 at open current potential for 5 min, followed by washing, transferring of protein modified GCE to blank electrolyte and recording of SW voltammogram.

2.3. Steady-state photolysis experiments

A home-made steady-state photolysis system was built by focusing a blue LED (443 ± 21 nm, 1 W) as selective excitation source of $Ru(bpy)_3^{2+}$. Photolysis of air-saturated solutions was performed with 10 μM BSA (or 1 mM aa's or 50 μg/mL polyamino acids) in presence of 20 μM $Ru(bpy)_3^{2+}$ in 50 mM Na-phosphate pH 7. Soft magnetic stirring of protein solution was done to avoid foam formation.

2.4. Urease activity measurements

2.4.1. In solution

0.05 μM urease was incubated in 100 μL of 1 M urea with 0.1% bromocresol purple for 5 min at 25 °C and absorption spectra were recorded on NanoDrop 1000 spectrophotometer with optical path length 1 mm [20,26].

2.4.2. At surface

Solid Ag-amalgam rod (Metrohm Switzerland) was prepared by brushing and dipping into mercury for several minutes and

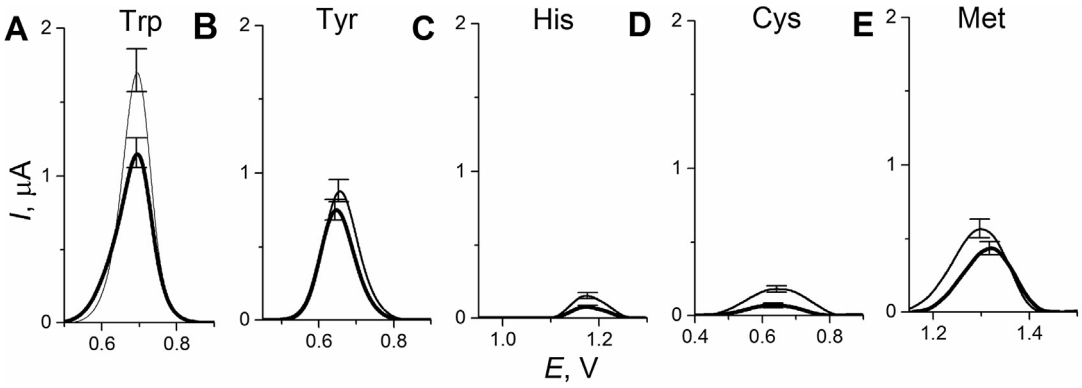

Fig. 1. *In situ* SW voltammograms of oxidation peaks of amino acids. 100 μM A. Trp, B. Tyr C. His D. Cys and E. 500 μM Met in presence of A. - D. 2 μM E. 10 μM $Ru(bpy)_3^{2+}$ after (thick line ———) or without (thin line ———) 60 min photosensitization with blue light. Amino acids were adsorbed for 300 s at 0.1 V at GCE and then SW voltammogram was recorded in 50 mM Na-phosphate pH 7. Amino acids solutions containing $Ru(bpy)_3^{2+}$ under dark conditions yielded the same peaks as amino acids in absence of $Ru(bpy)_3^{2+}$.