Biosensors and Bioelectronics 102 (2018) 464–469

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Nitration of tyrosine and its effect on DNA hybridization

Seda Nur Topkaya[a,*], Vasfiye Hazal Ozyurt[b], Arif E. Cetin[c], Semih Otles[b]

[a] Department of Analytical Chemistry, Faculty of Pharmacy, İzmir Katip Celebi University, Izmir, Turkey
[b] Department of Food Engineering, Faculty of Engineering, Ege University, Izmir, Turkey
[c] Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, USA

ARTICLE INFO

Keywords:
Electrochemical DNA biosensor
DNA hybridization
Protein nitration
3-Nitro-L-tyrosine
Tyrosine
Hybridization indicator

ABSTRACT

One major marker of nitrosative stress is the formation of 3-Nitrotyrosine (3-NT) from Tyrosine (Tyr) by adding a nitro group (-NO_2) with nitrating agents. Nitration of Tyr often causes loss of protein activity and is linked with many diseases. In this article, we detect 3-NT and discriminate it from Tyr with Differential Pulse Voltammetry (DPV) as it is a very important biomarker. We first examined redox (oxidation/reduction) properties and stability of 3-NT in detail. Second, we provided the Tyr and 3-NT discrimination with DPV and compared with the chromatography. We then explored the interaction of 3-NT and DNA oligonucleotides. Our findings demonstrate that 3-NT can be used as a new electrochemical indicator, which is able to detect hybridization of probe (single stranded DNA-ssDNA) and hybrid (double stranded DNA-dsDNA) both via 3-NT reduction and guanine oxidation signal changes at the same time. The signal differences enabled us to distinguish ssDNA and dsDNA without using a label or a tag. Moreover, we achieved to detect hybridization of DNA by using the reduction signal of 3-NT obtained at −0.4 V vs. Ag/AgCl. More importantly, we observed the changes of the reduction signals of 3-NT after the interaction of probe and hybrid sequences. We showed that 3-NT signal decreases more with hybrid than the probe. Our platform, for the first time, demonstrates the detection of hybridization both guanine oxidation and indicator reduction signal changes at the same time. Moreover, we, for the first time, demonstrated the interaction between 3-NT and DNA.

1. Introduction

Protein tyrosine nitration is a post-translational modification that occurs under the action of nitrating agents such as nitrate, nitrit and derivatives of peroxynitrit (Chen and Chen, 2012; Degendorfer et al., 2016). Aforesaid nitrating agents act as reactive compounds and help the formation of nitric oxide and peroxynitrit, which have been known as reactive nitrogen species (RNS). At physiological pH, RNS can react with a number of biomolecules such as DNA, proteins and lipids (Greenacre and Ischiropoulos, 2001). Tyrosine nitration changes the key properties of the amino acids such as redox potential, hydrophobicity and the values of pKa (Radi, 2013). RNS reacts with Tyrosine (Tyr) to form 3- Nitro-L-tyrosine (3-NT) replacing by hydrogen in the ortho position of the phenolic ring of the Tyr residues with a nitro group (-NO_2). 3-NT is therefore likely not a footprint for peroxynitrite alone but more generally a marker of nitrative stress. Chemical structures and the relationship between Tyr and 3-NT are shown in Fig. 1.

3-NT levels in biological matrices and fluids including plasma, serum, urine, cerebrospinal and synovial fluids have been associated with numerous physiological and pathological conditions such as immunological reactions, psychiatric disorders, cardiovascular and neurodegenerative diseases (Bolner et al., 2016; Daiber and Munzel, 2012; Zhao et al., 2014). A great deal of efforts has been spent to develop methods that accurately and sensitively quantify 3-NT since it can be used as a biomarker of nitrosative stress. The most preferred technique to determine the 3-NT is high-performance liquid chromatography (HPLC) (Teixeira et al., 2017) coupled to ultraviolet–visible (UV–Vis) (Erdal et al., 2008; Kilciksiz et al., 2011; Sharov et al., 2006) and fluorescence detector systems (FLD) (Guo et al., 2009). Recently, mass spectrometry (MS) or tandem mass spectrometry coupled with liquid chromatography (LC-MS/MS) is also introduced due to its ability to detect low concentration levels of analyte (Li et al., 2015; Wang et al., 2007). However, MS requires skilled operators and time consuming sample preparation steps. LC-MS/MS technique is also quite expensive due to the need of isotope labeled analogs of interest. Apart from the chromatographic based methods, other methods exist based on 3-NT detection in tissues and fluids with antibody-based methods, i.e., enzyme-linked immunosorbent assay (ELISA) (Sun et al., 2007; Weber et al., 2012). Generally, ELISA employs antibodies with tagged fluorescent dyes to measure the capture of target antigens. Both

* Corresponding author.
E-mail addresses: sedanur6@gmail.com, sedanur.topkaya@ikc.edu.tr (S.N. Topkaya).

https://doi.org/10.1016/j.bios.2017.11.061
Received 12 August 2017; Received in revised form 15 November 2017; Accepted 22 November 2017
Available online 23 November 2017
0956-5663/

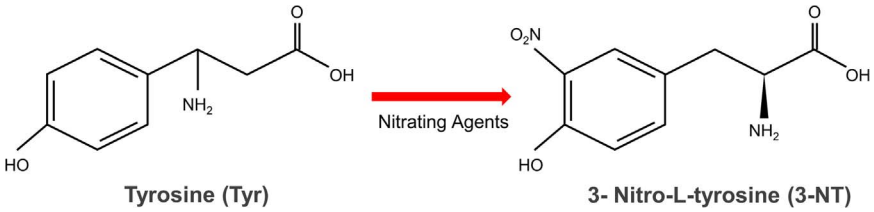

Fig. 1. Tyrosine conversion to 3-Nitro-L-tyrosine under the effect of nitrating agents. In the figure, nitrating agents are NO•; $ONOO^-$;NO^-; $ONOOCO_2^-$; NO_2Cl; NO_3^-; NO_2^-.

chromatographic and antibody-based detection methods have time-consuming extraction steps and/or require expensive standards.

The detection, identification and quantification of a biologically important target molecules and biomarkers, especially at low concentrations can be achieved with electrochemical based biosensor systems (Adhikari et al., 2017; Afkhami et al., 2017; Aghaei et al., 2017; Aghili et al., 2017; Alexander et al., 2017; Arevalo et al., 2017; Mahato et al., 2017). In that sense, electrochemical biosensors can enable highly sensitive, specific, rapid, low-cost and easy to handle detection. Another advantage of using electrochemical biosensors is that they can be miniaturized (lab-on-a-chip devices) and offer robust results compared to the classical analytical techniques such as immunohistochemistry or ELISA. Due to these unique advantages, electrochemical biosensors can successfully provide an efficient and reliable detection for the determination of 3-NT with high sensitivity in a cost-effective manner.

Herein, we aim to detect 3-NT and its interaction with DNA oligonucleotides by using its oxidation/reduction properties with voltammetry. In literature, there are very few studies on the detection of 3-NT with electrochemical techniques (Acar et al., 2016; Richards et al., 2006; Roy et al., 2015). In the present study, we first analyzed oxidation and reduction properties of Tyr and 3-NT and achieved the discrimination with Differential Pulse Voltammetry (DPV). Our electrochemical based results were compared with chromatography of Tyr and 3-NT discrimination, demonstrating that our method provides an easy-to-use (no extraction step), rapid (nearly 1 min) and cost effective detection. More importantly, in addition to clarifying redox properties of 3-NT and its discrimination from Tyr, we for the first time, showed 3-NT's interaction with DNA oligonucleotides. Briefly, electrodes were first activated to provide effective surface area. Then, probe sequences were immobilized onto the electrodes by dipping electrodes into the probe solution. Probe coated electrodes were later interacted with its target sequence to create hybrid form on the surface of electrodes. After obtaining the probe, hybrid (probe + target) on the electrode surfaces, we investigated the effect of 3-NT on DNA sequences. In order to detect the hybridization and interaction events, the changes in the oxidation signal of the guanine bases of DNA at approximately +1.00 V versus Ag/AgCl reference electrode were measured with DPV. Intrinsic electroactivity of guanine bases have been used as an indicator for the direct measurement of nucleic acids in a label-free assay (Dogan-Topal et al., 2009; Kilic et al., 2012; Topkaya et al., 2010). We observed that 3-NT behaves as a '**hybridization indicator**' due to its distinct electrochemical behavior to different strands of DNA. After interaction with 3-NT, guanine oxidation signals of probe signals decreased dramatically whereas hybrid signals remain almost unchanged. The signal differences enabled us to distinguish single stranded DNA (ssDNA-probe) and double stranded DNA (dsDNA-hybrid) without using a label or tag. Moreover, 3-NT reduction signals observed at nearly −0.4 V vs. Ag/AgCl were evaluated and optimized. In this study we, for the first time, showed the detection of hybridization of DNA by using the reduction signal of 3-NT. Interestingly, we observed the changes of the reduction signals of 3-NT after the interaction of probe and hybrid sequences. Hybridization indicators behave distinctly to different strands of DNA, which makes them an ideal candidate for detection of hybridization of DNA apart from the guanine signals. This feature is extremely important in lack of guanine bases during the investigation of sequence, such that indicators provide us an alternative route to detect hybridization.

Redox (oxidation/reduction) properties of 3-NT were first examined. Second, Tyr and 3-NT discrimination was provided with DPV and compared with HPLC. Third, the interaction of 3-NT and DNA oligonucleotides was explored. Last, hybridization was detected via guanine oxidation and 3-NT reduction signals at the same time. Our findings introduced **a new hybridization indicator,** 3-NT to the literature.

2. Materials and methods

2.1. Instruments

DPV measurements were carried out using μ-AUTOLAB Type III-Electrochemical Analysis System (Eco Chemie, Netherlands). Three electrode system consists of a pencil graphite electrode (PGE) as the working electrode, a reference electrode (Ag/AgCl, Model RE-1 BAS, USA), and a platinum wire as the auxiliary electrode. The pencil graphite leads are composite materials containing graphite (~ 65%), clay (~ 30%), and a binder (wax, resins, or high polymer). HB type pencil leads were used which contain equal portions of graphite and clay. The surface area was calculated as 0.255 cm^2 for PGE. HB pencil leads with a length of 60 mm and a diameter of 0.5 mm were employed. Pencil Model T 0.5 mm (Rotring, Germany), was used as a holder for pencil lead (Tombo, Japan), which were purchased from a local bookstore.

Agilent 1200 HPLC apparatus (Agilent Technologies, Germany) equipped with a diode array detector (DAD), a quaternary elution pump, and auto sampler injection system and a temperature-controlled column oven were used. An Inertsil ODS-3 RP-HPLC column (5 μm, 150 × 4.6 mm) and a guard column (10 × 4.6 mm) filled with the same material was chosen.

2.2. Chemicals

Tyrosine ($C_9H_{11}NO_3$) and 3-NT ($C_9H_{10}N_2O_5$) were supplied from Sigma. Stock solution of Tyr and 3-NT (1 mg/mL) was prepared by dissolving a weighed amount in ultrapure water. Diluted concentrations of Tyr and 3-NT were prepared in daily with 0.05 M phosphate buffer containing 20 mM NaCl (PBS, pH 7.4). Other chemicals in analytical reagent grade were supplied from Sigma and Merck.

2.3. Oligonucleotides

HPLC-purified oligonucleotides were purchased as lyophilized powders from TIB-MOLBIOL (Germany). Their base sequences are as follows:

Probe: 5′-TTC GGG GTG TAG CGG TCG TC-3′

Target: 5′-GAC GAC CGC TAC ACC CCG AA-3′

The oligonucleotides stock solutions were prepared in ultrapure water and stored at −20 °C. Diluted solutions of probe were prepared using 0.5 M acetate buffer solution containing 20 mM NaCl (ACB, pH:4.8) and diluted target solutions were prepared using PBS.