Analytical Chemistry: An Indian Journal

ISSN: 0974-7419

Opinion| Vol22Iss1

Investigation of the Reaction of Peroxynitrite with Myoglobin for Meat Extract Samples using Cobalt Phthalocyanine-Modified Screen-Printed Carbon Electrodes and a Flow Injection Analysis System

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Received: January 6, 2022, Manuscript No. tsac-22-71448; **Editor assigned:** January 7, 2022, PreQC No. tsac-22-71448 (PQ); **Reviewed:** January 21, 2022, QC No. tsac-22-71448 (Q); **Revised:** January 22, 2022, Manuscript No. tsac-22-71448 (R); **Published date**: January 26, 2022. doi: 10.37532/0974-7419.2022.22(1).177

Abstract

The effects of Reactive Oxygen/Nitrogen Species (ROS/RNS) on cellular responses to stress, cell growth, cell death, cancer, ageing, and male infertility are significant. Monitoring the quality and freshness of raw meat is also crucial for both the food business and consumers. A variety of indicators, including as discoloration, rancidity, and taste changes, might point to meat that has been altered. The scavenging activity of myoglobin against RNS (like Peroxynitrite, PON) is one mechanism of modification. Using Cobalt Phthalocyanine (CoPc) as a straightforward, affordable, highly thermally stable, and biomimetic catalyst, this paper describes the development of an electrochemical PON sensor and the use of this sensor, which is based on Screen-Printed Carbon Electrodes (SPCEs), to analyse meat extract samples using Flow Injection Analysis (FIA). Peroxynitrite reduction is mediated by CoPc. SEM, FTIR, and cyclic voltametry were used to characterize the surface of the modified electrode. Both UV-Vis and chronoamperometry were used to investigate the interaction of PON with myoglobin (at 0.1 V, using the FIA system). The electrode was calibrated using these equations: Ired (n_A)=6.313 CPON (M)+17.469; (R=0.9938). The linear range was 3 M-180 M, and the computed LOD was 2.37 M. Pre-treatment (electro-reduction of the CoPc deposited layer, at 0.3 V, for 60 s) can further enhance the performance of the electrode. In a very selective, sensitive, and repeatable manner, this might assist us in monitoring and quantifying how much PON was destroyed and when meat extracts are treated with various PON concentrations.

Introduction

Monitoring the quality and freshness of raw meat is crucial for both the food business and consumers. A variety of indicators, including as discoloration, rancidity, and taste changes, might point to meat that has been altered. The scavenging ability of myoglobin toward nitro-oxidative compounds is one mechanism of change (such as Peroxynitrite, PON). Superoxide anion (O_2) is removed by Super-Oxide Dismutase (SOD) at a rate of 1 Ms^{-1} to 2 Ms^{-1}), and NO and O_2 react quicker (10 Ms^{-1}) in order to compete with SOD for O_2, directing to the synthesis of the highly reactive compound known as oxoperoxonitrate Anion of Peroxynitrite (PON). Peroxynitrite's (ONOOH) protonated form has an extraordinarily quick membrane permeabilization. However, in neutral and acidic condition, it disintegrates because peroxynitrous acid breaks down into nitrites, nitrates, and other oxygen species. The peroxynitrite content, temperature, antioxidant status, and presence of scavenging species all affect the breakdown process and speeds. The capacity of iron to transition from Fe^{2+} to Fe^{3+} oxidation state has a significant impact on the tissue's colour. Another crucial component is the heme center's binding location. The coordination site is vacant and the flesh is purple in deoxymyoglobin, $MbFe^{2+}$. When oxygen is bound to the sixth coordination site, oxymyoglobin ($MbFe^{2+}O_2$) is formed, but

Citation: Jason F. Investigation of the Reaction of Peroxynitrite with Myoglobin for Meat Extract Samples using Cobalt Phthalocyanine-Modified Screen-Printed Carbon Electrodes and a Flow Injection Analysis System. Anal Chem Ind J. 2022;22(1):177.

the oxidation state remains unchanged. development of metmyoglobin, Oxidation of lipids and proteins can change the taste of $MbFe^{3+}(OH_2)$, an Fe^{3+}centre with an empty coordination site and brownish hue. The quality of the meat has a significant influence on customers and the food business. Myoglobin oxidation processes are irreversible if metmyoglobin ($MbFe^{3+}(OH_2)$ reducing enzyme systems are absent from meat after slaughter. The variations in hue are another indication of these processes. Since NO (nitric oxide) may connect to the iron ion in a manner similar to how the oxygen molecule does, adding nitrites to High performance liquid chromatography, chemiluminescence, fluorescence spectroscopy, UV-Visible absorbance spectroscopy, electron spin resonance, and electrochemistry are the most frequently used techniques to detect peroxynitrite. In batch analysis, these techniques often employ antioxidants like resveratrol, polyphenols, or catechins, but they may also be used with flow injection analysis, for instance by infusing antioxidants that can quench the peroxynitrite. The microfluidic injection analysis offers a number of benefits, including the presence of laminar flow with no dilution effects, the requirement for a small volume of analyte (less than 150 L), miniaturisation, the potential for real-time continuous monitoring (process control), a quicker and more sensitive response, or the potential for automated processes. Raw meat helps maintain the colour of the meat's vivid red hue. Other chemicals that break down peroxynitrite include nitrates and nitrites. To better understand other crucial concerns, such how cancer develops, it would be interesting to create an electrochemical sensor that can distinguish between PON and other interfering species in biological tissues. In the literature, a number of electrochemical sensors are discussed. One of the articles that received the most citations described the electrochemical oxidation of peroxynitrite and its detection at a single cell's surface. The authors analyzed the sensitivity and found that they were all oxidations that took place at potentials greater than 0.5 V, making the electrode more vulnerable to signal interferences since other molecules are more likely to be oxidized at these potentials. Using a chemically modified electrode, batch reduction of peroxynitrite is only described in a very small number of papers. Using the electro-oxidation of the metallic site, Mn-pDPB (Manganese-[Poly-2,5-Di-(2-Thienyl)-1h-Pyrrole)-1-(P-Benzoicacid)] catalyzed the reduction of peroxynitrite at 0.2 V (Sensitivity: 157.0 nM, LOD: 1.9 nM), and selectivity and stability are provided by coating the at 0.35 V, manganese(III)- paracyclophenylporphyrin-modified carbon microfibre electrodes accelerate the electro-reduction of liberated PON from human endothelial cells (LOD: 1 nM). Using a platinum electrode modified with electropolymerized manganese tetraaminophthalocyanine was used to reduce produced PON in an alkaline solution (pH 12) at -0.45 V (Sensitivity: 14.6 $nAmM^{-1}$, LOD: 5 M).

Conclusion

For the direct detection of peroxynitrite, our label-free electrochemical approach suggests depositing cobalt phtalocyanine on the screen-printed carbon electrode. This approach is straightforward, perceptive, incredibly selective, quick, and economical. The SPCE/CoPc electrode was thoroughly evaluated and made to work best for peroxynitrite detection. The linear range of this electrode is between 3 µm and 180 µm, and the limit of detection is 2.37 µm, making it appropriate for biological matrices. Additionally, before use, this electrode may be further optimised by having the CoPc layer that has been placed on it reduced (either chemically using sodium borohydride or electrochemically).