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Suggestion a catalytic interaction between homocysteine as a thiolic amino acid and vinylferrocene as an organometallic mediator at a control potential

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Abstract: New interaction between vinylferrocene (VF) and homocysteine (Hcy) was investigated in this work. At control potential, an electrocatalytic interaction occurs between Hcy and VF in aqueous buffer solution. Kinetic parameters such as electron transfer coefficient (α) and heterogeneous rate constant (k_h) for Hcy were also determined using electrochemical approaches.

Keywords: Homocysteine, Organometallic mediator, Voltammetric interaction.

Introduction

The biochemistry of organosulfur compounds in microorganisms, plants, and animals has been extensively studied [1]. Much less is known about the biotransformations of organic sulfur compounds in natural environments.

Homocysteine (as a thiol amino acid) was discovered by serendipity in 1934 as a byproduct of the digestion of methionine with hydriodic acid, a procedure used then for the determination of protein methionine [2]. The determination of Hcy has gained high interest within the biomedical community over recent years as it is a major biomarker for a wide range of diseases [3]. The distribution of Hcy among different tissues, cells, and intracellular compartments is an important factor affecting many physiological concentrations. In biological systems, Hcy is usually bound in a disulfide linkage; the usual level of the free unbound Hcy species is approximately 1-2% of the total Hcy concentration [4].

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However, in patients with genetic disorders of Hcy metabolism or patients suffering from cardiovascular diseases, the concentration of free Hcy increases [5] and, therefore, its monitoring can be crucial to the medical community as a cardiacmarker. Several methods have been proposed for the determination of Hcy that include gas chromatography-mass spectroscopy (GC/MS) [6], HPLC (or capillary electrophoresis) with fluorescent [7], laser-induced fluorescent [8], mass spectrometric [9], and electrochemical methods [10–12].

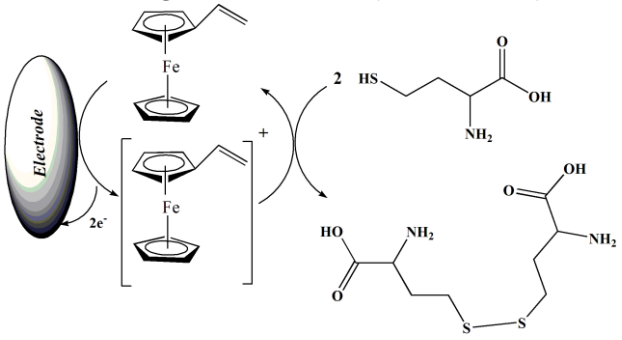
As the functional group of the amino acid Hcy, the thiol group plays a very important role in biology. When the thiol groups of two Hcy residues (as in monomers or constituent units) are brought near each other in the course of protein folding, an oxidation reaction can generate a Hcy unit with a disulfide bond (-S-S-). Disulfide bonds can contribute to a protein's tertiary structure if the Homocysteines are part of the same peptide chain, or contribute to the quaternary structure of multi-unit proteins by forming fairly strong covalent bonds between different peptide chains.

In this study we describe an electrocatalytic interaction between VF and Hcy as an electrochemical strategy for determination of Hcy in biological samples.

Results and discussion

Mechanism suggestion:

To our knowledge, there is no any report for investigation of interaction between VF and Hcy under electrochemical process. Therefore, we suggest a mechanism for this process in a voltammetric condition (under control potential in electrochemical cell) and in continuous determine kinetic and thermodynamic parameter using this mechanism (see scheme **1**).



Scheme 1: Propose mechanism for electrochemical interaction between vinylferrocene and homocysteine.

Catalytic effect:

Figure **1** depicts the cyclic voltammetric responses from the electrochemical oxidation of 1.0 mM Hcy at VFCNTPE (curve c), VF modified carbon paste electrode (VFCPE) (curve b), carbon nanotubes paste electrode (CNTPE) (curve d), and bare CPE (curve e). As can be seen, the anodic peak potential for the oxidation of Hcy at VFCNTPE (curve c) and at VFCPE (curve b) is about 380 mV, whereas at CNTPE (curve d) the peak potential is about 710 mV, and at the bare CPE (curve e), the peak potential is about 750 mV. From these results, it is concluded that the best electrocatalytic effect for Hcy oxidation is observed at VFCNTPE (curve c). For example, the results show that the peak potential of Hcy oxidation at VFCNTPE (curve c) shifted by about 330 and 370 mV toward the negative values compared with that at CNTPE (curve d) and at bare CPE (curve e), respectively. Similarly, when we compared the oxidation of Hcy at VFCNTPE (curve c) and VFCPE (curve b), there is an enhancement of the anodic peak current at VFCNTPE relative to that value obtained at VFCPE. In other words, the data obtained clearly shows that the combination of carbon nanotubes and mediator definitely improved the characteristics of Hcy oxidation. VFCNTPE in 0.1 M phosphate buffer (pH 7.0), without Hcy, exhibits a well-behaved redox reaction (curve a), and upon the addition of 1.0 mM Hcy, the anodic peak current of mediator was greatly increased. In addition, the corresponding cathodic peak disappeared on the reverse scan of the potential (curve c). This behavior is typical of that expected for electrocatalysis at chemically modified electrodes.

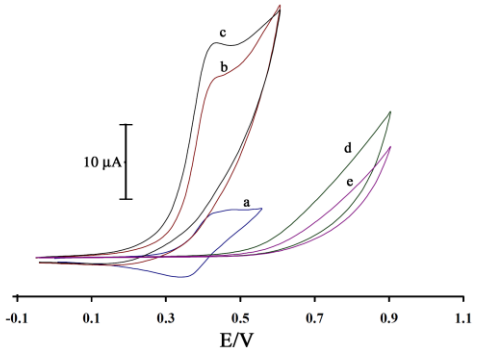


Figure 1: Cyclic voltammograms of 0.1 mol L^{-1} PBS (pH 7.0) with a scan rate of 20 mV s^{-1} for: (a) in the absence and (c) in the presence of 1.0 mM Hcy at VFCNTPE; (c) is as (b) at VFCPE; (d) as a (c) and (e) as (b) at CNTPE and CPE, respectively.

To obtain further information on the rate-determining step, a Tafel plot was developed for VFCNTPE using the data derived from the raising part of the current–voltage curve (Figure **2**). The

Tafel slope was found to be 0.1403 V (Figure **2**, inset c), which indicates that transfer coefficient (α) is about 0.58.

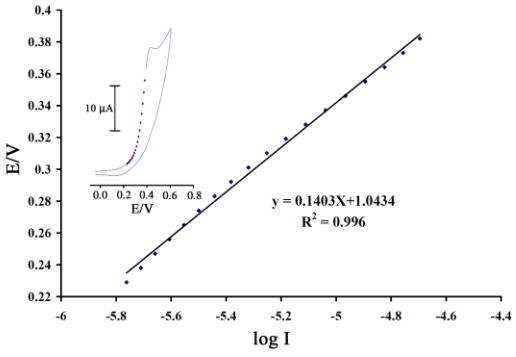


Figure 2: Tafel plot for VFCNTPE in 0.1 mol L^{-1} PBS (pH 7.0) at a scan rate of 20 mV s^{-1} in the presence of 1.0 mM Hcy.

pH optimization:

It is well known that the electrochemical behavior of Hcy is dependent on pH value of the solution, whereas the electrochemical properties of Fc/Fc+ redox couple

are pH independent. Therefore, optimization of the solution pH seems to be necessary. Thus, we studied the electrochemical behavior of Hcy in 0.1 M PBS in different pH values (4.0<pH<8.0) at the surface of VFCNTPE using cyclic voltammetry. Results show, the anodic peak current for electrooxidation of Hcy reached to a maximum value at pH 7.0. Therefore, pH 7.0 was chosen as the optimum pH for electrocatalysis of Hcy oxidation at the surface of VFCNTPE. Hence, all electrochemical experiments were done at this pH.

The rate constant for the chemical reaction between Hcy and redox sites in VFCNTPE, k_h, can be evaluated by chronoamperometry according to the method of Galus [13]:

$$I_C/I_L = \pi^{1/2} \gamma^{1/2} = \pi^{1/2} (K_h C_b t)^{1/2} \quad (1)$$

where I_C is the catalytic current of Hcy at VFCNTPE, I_L the limited current in the absence of Hcy, and t is the time elapsed (s). Based on the slope of the I_C/I_L versus $t^{1/2}$ plots (Figure **3**), k_h can be obtained for a given Hcy concentration. Using the values of the slopes, the average value of k_h was found to be $k=1.6\times10^4$ mol^{-1} L s^{-1}.

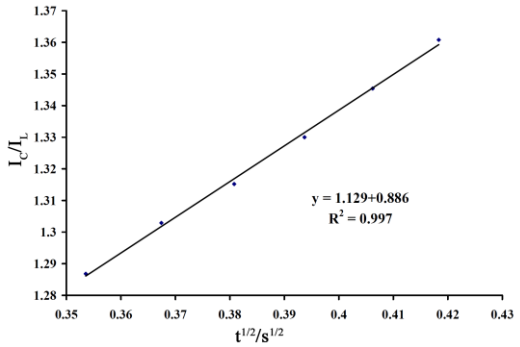


Figure 3: Dependence of I_c/I_L on the $t^{1/2}$ for 1.0 mM Hcy.

Dynamic range and limit of detection:

Differential pulse voltammetry was used to determine the concentration of Hcy. Responses were linear with Hcy concentrations ranging from 0.1×10^{-6} to 220.0×10^{-6} M and a current sensitivity of 0.03703 μA/μM. The detection limit (3σ) was 0.06 μM.

Conclusion

In this study we describe as new strategy for interaction between vinylferrocene as an organometallic mediator with Hcy as an amino acid. This interaction was used as a suitable method for determination of Hcy in trace level.

Experimental

Chemicals:

All chemicals used were of analytical reagent grade purchased from Merck (Darmstadt, Germany) unless otherwise stated. Doubly distilled water was used throughout.

Phosphate buffer (sodium dihydrogen phosphate and disodium monohydrogen phosphate plus sodium hydroxide, 0.1 mol L^{-1}) solutions (PBS) with different pH values were used.

High viscosity paraffin (d = 0.88 kg L^{-1}) from Merck was used as the pasting liquid for the preparation of the carbon paste electrodes. Spectrally pure graphite powder (particle size<50 μm) from Merck and multiwall carbon nanotubes (>90% MWNT basis, d × l = (100 – 80 nm) × (5 – 9 μm) from Fluka were used as the substrate for the preparation of the electrodes.

Apparatus:

Cyclic voltammetry (CV), and chronoamperometry were performed in an analytical system, Autolab with PGSTAT 302N (Eco Chemie, the Netherlands). The system was run on a PC using GPES software. A conventional three-electrode cell assembly consisting of a platinum wire as an auxiliary electrode and an $Ag/AgCl/KCl_{sat}$ electrode as a reference electrode was used. The working electrode was either an unmodified carbon nanotubes paste electrode, or a vinylferrocene modified carbon nanotubes paste electrode (VFCNTPE).

Preparation of the electrode:

2.0 mg of VF was hand-mixed with 88 mg of graphite powder and 10 mg of multiwall carbon nanotubes in a mortar and pestle. Using a syringe, 0.50 g of paraffin was added to the mixture and mixed well for 40 min until a uniformly-wetted paste was obtained. The paste was then packed into a glass tube. Electrical contact was made by pushing a copper wire down the glass tube into the back of the mixture. When necessary, a new surface was obtained by pushing an excess of the paste out of the tube and polishing it on a weighing paper. The unmodified carbon paste electrode (CPE) was prepared in the same way without adding VF and carbon nanotubes to the mixture to be used for comparison purposes.

Preparation of real samples:

Urine samples were stored in a refrigerator immediately after collection. Ten milliliters of the sample was centrifuged for 5 minutes at 1500 rpm. The supernatant was diluted 100 times with universal buffer pH = 7.0. The solution was transferred into the voltammetric cell to be analyzed without any further

pretreatment. Standard addition method was used for the determination of Hcy in real samples.

Acknowledgement

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