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In memoriam prof. dr. Liviu Oniciu

ELECTROCHEMISTRY OF IRON (III) PROTOPORPHYRIN (IX) SOLUTION AT GRAPHITE ELECTRODE

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ABSTRACT. Cyclic voltammmetry was used to investigate the electrochemical properties of iron (III) protoporphyrin (IX) (hemin, Fe(III)P) dissolved in TRIS buffer at a graphite electrode. A quasi-reversible single electron transfer attributed to the Fe(III)P/Fe(II)P redox process controlled by diffusion was identified. The pH influence on the electrochemical activity of hemin and its electrocatalytic behaviour towards nitrite reduction was also investigated and demonstrated.

Keywords: *iron (III) protoporphyrin (IX), nitrite, cyclic voltammetry.*

INTRODUCTION

Nitrite is an important source of nitrogen in green plants and its complete reduction to ammonia involves the overall transfer of six electrons [1]. The reduction of nitrate and nitrite has gained renewed attention in view of its relevance to pollution control due to excessive use in fertilizers, detergents, industrial processes and food technologies. Also, the control of water quality is important to avoid contamination of food produced when water is used as a raw material. Electrochemical reduction catalysis can be advantageously applied to the treatment of industrial wastewater, whereby nitrate species are transformed into harmless reduction products from various cathodic materials and solutions of different compositions. Generally, however, the electrochemical reactions of interest have been found to proceed at potentials substantially more negative than their thermodynamic values with low current density, providing evidence that their energies of activation are very high [2].

Organo-iron derivatives have been identified as intermediates in several biological processes. The main motivation for the investigation of iron-porphyrins' redox properties has been to establish their correlation with

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structure–function relationships of complex homoproteins. Due to electrochemical reversibility of iron-porphyrin derivatives, they can be used as electron transfer mediator for modification of different electrode materials, and preparations of chemically modified electrodes with these compounds have received great interest in the field of electroanalysis. Different supporting carbon materials have been used to disperse and stabilize electron transfer mediators, due to their low background currents, wide potential windows, chemical inertness and low costs [3]. Iron-porphyrins are also well recognized for their excellent electrocatalytic properties toward the detection of many important analytes, such as nitric oxide [4], neurotransmitters [5], O_2 [6], hydrogen peroxide [7], nitrite [8], superoxide [9], sulfur oxoanions [10], tryptophan and its derivatives [11 - 12].

Hemin (iron protoporphyrin IX) (Fe(III)P) is a naturally occurring iron-porphyrin complex possessing catalytic function [7] and as consequence is the active center of the family of heme-proteins, such as b-type cytochromes, peroxidase, myoglobin and hemoglobin [9, 13]. Its capacity to mimics the catalytic properties of enzymes lead to an intensive research activity of this small molecule. Hemin dissolved in an aqueous solution, adsorbed on an electrode surface [14] or immobilized on ion-exchange resins, zeolites, silica, clays, in polymer or lipid film on an electrode surface was studied from electrochemically point of view [15, 16]. Also, it was used as catalyst for dioxigen [6, 17 - 18], nitrite [1], hydrogen peroxide [19].

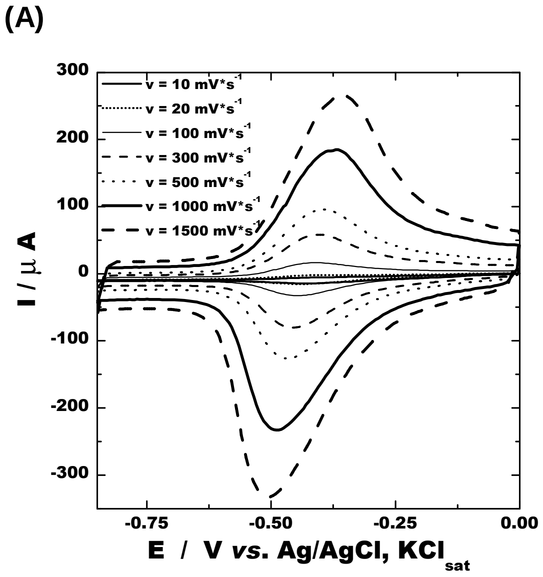
The aim of this paper was a fully electrochemically characterization of the hemin in solution and the identification of its capacity to electrocatalyse the nitrite reduction.

RESULTS AND DISCUSSION

Electrochemical behaviour of hemin on graphite electrode

Figure 1A shows a set of cyclic voltammograms obtained at different scan rates, when the graphite electrode was immersed in the hemin solution. A well formed and similarly shaped pairs of peaks corresponding to the single electron transfer into the Fe(III)P/Fe(II)P redox couple is observed at $E_{p,a} = E_{p,c} = -0.42$ V *vs*. Ag/AgCl, KCl_{sat} ($\Delta E_p = 0$), only at 10 mVs^{-1} as in the case of the reversible processes. The variation of the scan rate between 100 and 1000 mVs^{-1} leads to an exponentially increasing of peak separation ΔE_p (calculated as E_{pa}- E_{pc}), as it is observed for the quasi-reversible systems controlled by diffusion (figure 1B).

At scan rates ranging from 100-1000 mVs^{-1} the peaks currents (I_p) increase linearly with the scan rate (v) and not with $v^{1/2}$ (slope of log I_p *vs.* log v between 0.85 and 1). This indicates that the redox reaction is a surface process, due to the slow adsorption of the hemin on the electrode surface during the measurements.



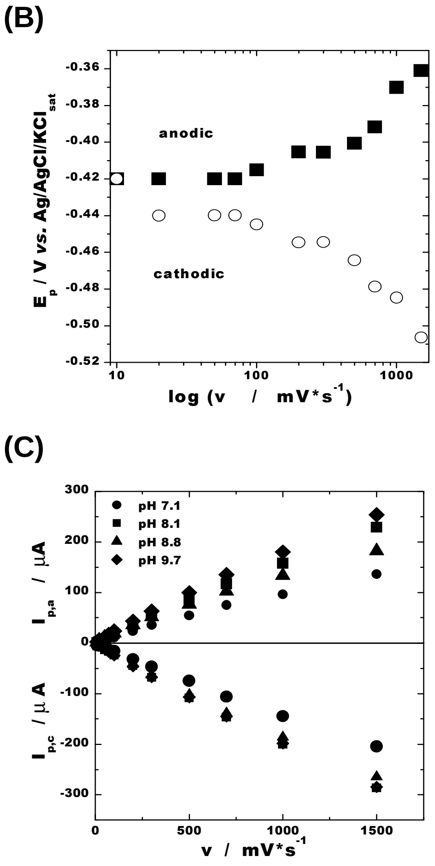


Figure 1. Cyclic voltammograms of 0.5 mM Fe(III)P solution at a graphite electrode **(A)**. The influence of the scan rate on the peak potentials **(B)** and on the peak current **(C)**. Experimental conditions: electrolyte, 0.05 M TRIS buffer (pH 8) solution; starting potential, -0.85 V *vs*. Ag/AgCl, KClsat, deoxygenated solution.

The effect of pH on the electrochemical behavior of hemin on graphite electrode was studied in different buffer solutions (pH 6.5 - 10) in the absence of oxygen. The pairs of well-defined redox peaks of hemin are strong pH dependent. The current intensity of peaks has a maximum value in the range pH 8-8.5, in spite of the potential scan rate (Figure 2).

Both reduction and oxidation peak potentials of Fe(III)P/Fe(II)P redox couple shifted negatively with an increase in pH (results not shown). The pH dependencies of the formal peak potentials in the studied pH range can be expressed as follows: $E^{0'} = -38,55 - 47.95pH$ (R = 0.995, n = 7, v = 300 mVs^{-1}) or $E^{0'} = -70.82 - 44.27pH$ (R = 0.995, n = 8, v = 500 mVs^{-1}). (The formal potential is the mean between E_{pa} and E_{pc}).

As observed in similar cases [12, 16] the slopes of $E^{0'}$ - pH dependence are reasonably close to the theoretical value of −59.1 mV pH^{-1} at 25 ^{0}C for a reversible one proton coupled with one electron redox reaction process in accordance with the following equation:

$$(H_2O)(OH)Fe(III)P + H^+ + e^- \rightleftharpoons (H_2O)_2Fe(II)P \qquad (1)$$

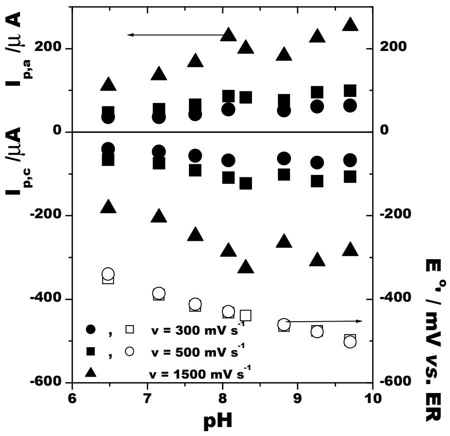


Figure 2. The current peak and the formal peak potential dependences on the pH of Fe(III)P solution. Experimental conditions: see figure 1.

It is possible that the pH dependency of $E^{o'}$ of Fe(III)P is due to changes in the ligation of the metal. In weak acidic and neutral pH range, the iron is coordinated to water molecules and hydroxyl ions. At basic pH, it is difficult to assign a mechanism for the proton/electron transfer coupling. If we consider a low surface coverage of the Fe(III)P on the electrode, we can assume that there is small interaction between adjacent porphyrins. In this case the formation of monomeric Fe(III)P$(OH)_2$ would explain the coupling between electron and proton transfer [16].

Due to stability, electrochemical reversibility and high electron transfer rate constant of Fe(III)/Fe(II) redox couple at graphite electrode, it can be used as a mediator to shuttle electrons between electrodes and analyte molecules. To assess the electrocatalytic properties of hemin, its electrocatalytic activity towards nitrite reduction was examinated. In order to test this ability of the studied molecule, cyclic voltammograms were obtained in the presence and absence of nitrite and/or hemin in Tris buffer solution (pH 8).

As shown in figure 3, in the presence of hemin and different concentrations of nitrite, a cathodic peak at ~ -0.8 V accompanied by a current increasing, indicates a strong catalytic activity of hemin toward these analyte. At the bare surface of the graphite electrode, in the absence of hemin and in the presence of nitrite no response was observed. The same behavior was observed for the electroreduction of chlorate and iodate at multi-walled carbon nanotubes (MWCNTs) and Fe(III)P-MWCNTs-modified electrodes [12].

Casella and al. [2] have demonstrated that a positive value of the switch potential (~ 0.5 V) is important for the appearance of the nitrite reduction peak. So it can be considered that the over all reduction process involves a preliminary adsorption process of analyte on the electrode surface, and the adsorbed complex undergoes a subsequent multi-step reduction process in the negative region of potentials between –0.3 and –1.3 V.

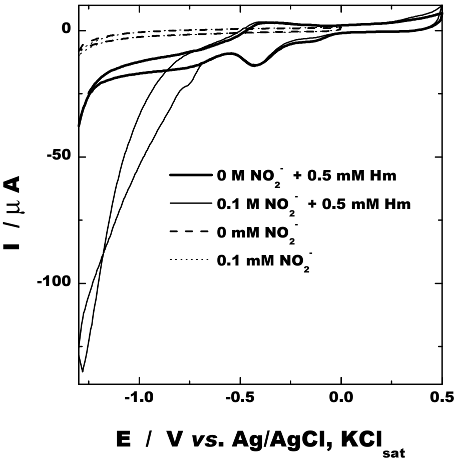


Figure 3. Electrocatalytic effect of 0.5 mM Fe(III)P on the nitrite reduction. Experimental conditions: electrolyte, 0.05 M TRIS buffer (pH 8) solution; scan rate, 20 mVs^{-1}; starting potential, -1.3 V *vs.* Ag/AgCl, KCl_{sat}; deoxygenated solution.

These fore, in all the reported studies [20 -22], it was suggested that the first step of the catalytic process at pH 7.4, involves the formation of an iron-nitrosyl complex as a consequence of the metal-centered Fe(III)P/Fe(II)P reduction followed by nitrite binding, according to equations 2-3:

$$\mathrm{Fe(III)P} + e^- \rightarrow \mathrm{Fe(II)P} \qquad (2)$$

$$\mathrm{Fe(II)P} + \mathrm{HONO} + H^+ \rightarrow [\mathrm{Fe(II)P(NO^+)}]^+ + H_2O \qquad (3)$$

These steps are then followed by the reduction of the iron-nitrosyl adduct, according to equation 4:

$$[\mathrm{Fe(II)P(NO^+)}]^+ + e^- \rightarrow [\mathrm{Fe(II)P(NO\bullet)}] \qquad (4)$$

It should be noted that no clear evidence to fully characterize the above-cited reduction process and no accurate overall catalytic process was established in the literature. Only a few attempts were reported to model a probable mechanism of reduction of nitrite to ammonia by iron porphyrins [1].

CONCLUSIONS

The electrochemical investigation by cyclic voltammetry of the iron (III) protoporphyrin (IX) reveals a quasi-reversible behaviour, corresponding to a one-electron transfer redox process at a graphite electrode. The influence of the buffer pH on the peak current and $E^{0'}$ of hemin was also, investigated.

The electrocatalytic ability of hemin towards nitrite reduction was evidenced by recording cyclic voltammograms on graphite electrode in the presence and absence of nitrite in buffer solution. A decrease in overpotential and enhancement of peak current for nitrite reduction indicates strong catalytic activity of porphyrin toward these analyte.

EXPERIMENTAL SECTION

Reagent and materials

Iron (III) protoporphyrin (IX) chloride, $NaNO_2$, HCl, NaOH and TRIS chloride were purchase from Sigma. All reagents were of analytical grade and were used as received, without further purification.

A 0.5 mM stock solution was prepared by dissolving the appropriate amount of hemin in 0.05 M TRIS chloride buffer (pH 10.5). A 1M nitrite solution was prepared in above 0.05 M Tris. The pH adjustment of solution was achieved by using HCl and NaOH. Deionized water was used for preparing all solutions.

Apparatus

Cyclic voltammetric investigations were carried out on a computer controlled AMEL 433 trace analyser (*AMEL, Milan, Italy*).

All measurements were done using a standard single-compartment three electrode cell equipped with a platinum counter electrode, an Ag/AgCl, KCl_{sat} reference electrode (*Radiometer*, *France*) and a spectral graphite (3 mm diameter) (*Ringsdorff-Werke, Gmbh, Bonn-Bad Godesberg, Germany*) working electrode. The surface of the graphite electrode was polished with 600, 1000, and 2000, grit SiC emery paper, washed with water and then sonicated in water. All experiments were performed at room temperature. The oxygen was purged from the electrolyte solutions by bubbling with high purified argon and all experiments are done under this inert atmosphere.

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