ELECTROCHEMICAL STUDIES OF INTERACTIONS BETWEEN Fe(II)/Fe(III) AND AMINO ACIDS USING FERROCENE-MODIFIED CARBON PASTE ELECTRODE

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Abstract: The electrochemical behavior of an Fe(II)/Fe(III) redox couple in the presence of various selected amino acids has been studied using ferrocene-modified carbon paste electrode at pH = 7.4. Because of Fe(II)/Fe(III) solubility issues at physiological pH, ferrocene was used as a source of iron. Anodic oxidation of iron (pH = 7.2) occurred at 0.356 V and cathodic oxidation at 0.231 V, both *vs* Ag|AgCl. Treatment of the voltammetric data showed that it was a purely diffusion-controlled reaction with the involvement of one electron. After addition of amino acids, potential shifts and current changes can be observed on the voltammograms. Cyclic voltammetry experiments revealed the capability of amino acids to change the electrochemical behavior of the Fe(II)/Fe(III) redox couple.

Key words: iron, amino acid, cyclic voltammetry, ferrocene, carbon paste electrode

1. Introduction

Iron has long been implicated in neurodegenerative disease through its redox transitions *in vivo*. The consequential generation of oxygen free radicals can further induce oxidative stress in tissues (WINTERBOURN, 1995; LLOYD *et al*., 1997; ZATTA, 2003; VALKO *et al.*, 2005). Abnormally high levels of iron and oxidative stress have been found in neurodegenerative disorders such as Alzheimer and Parkinson diseases, multiple system atrophy, and progressive supranuclear palsy (SMITH *et al*., 1997; SAYRE *et al*., 2000; ARREGUIN *et al.*, 2009).

This evidence for misregulation of iron concentrations in neurodegenerative diseases highlights the need to understand interactions of endogenous biological important substances, like amino acids, with iron (GARCÍA et al., 2012).

The most important mammalian iron storage protein is ferritin, widely distributed in nature; it sequesters a large amount of iron in the protein interior. The diameter of the roughly spherical protein interior is about 6 nm and, when filled to capacity, the crystalline polymeric iron core can accommodate up to 4500 iron atoms (WATT *et al*., 1985; JAMESON and LINERT, 1997; BOU-ABDALLAH, 2010).

Unfortunately, a limitation of the electrochemical approach, in the case of studying interactions between Fe(II)/Fe(III) and amino acids is that it cannot be applied at physiological pH values because of the insolubility of Fe(II)/Fe(III) above pH 4.0.

Carbon paste electrodes (CPEs) are inherent part of electroanalytical chemistry due to their unique properties such easy surface renewing or applicability in inorganic, organic and biological analysis (ŠVANCARA *et al.*, 2012). Carbon pastes can easily

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be modified by addition of a solid compound and then, they are called "modified CPEs". Ferrocene (Fc) and its derivatives are widely used in electrochemistry because of their good stability in solution and rapid response to many substances (KAMYABI and AGHAJANLOO, 2009).

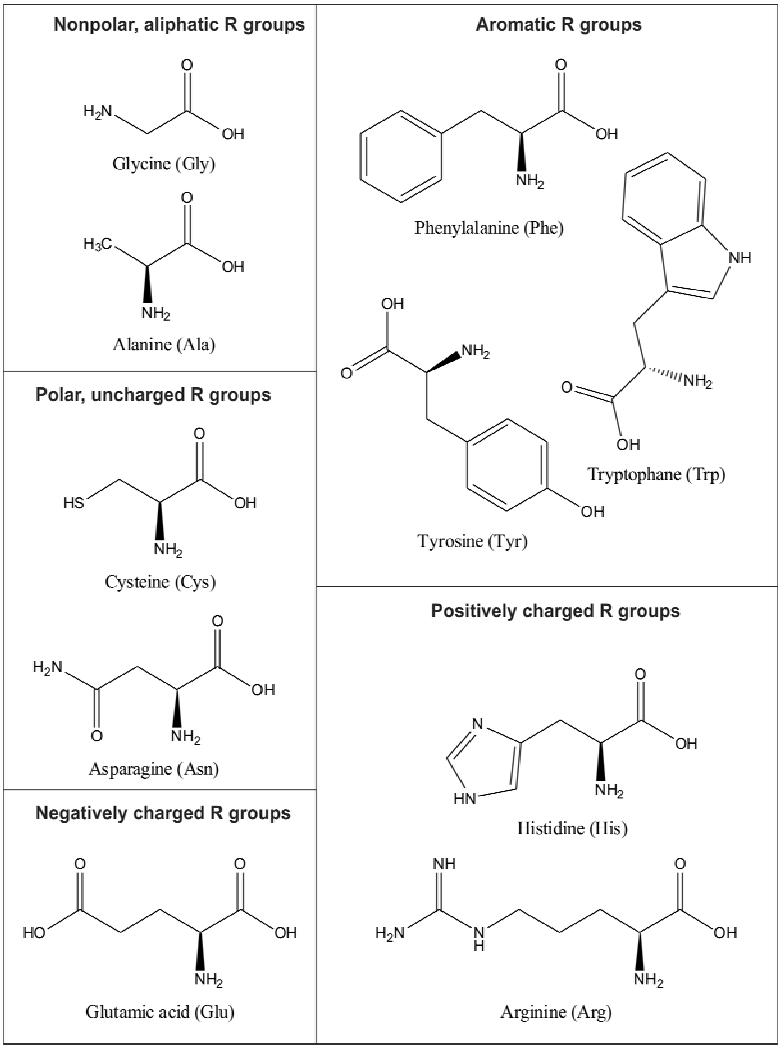

Fig. 1. Selected amino acids classified according to their chemical properties.

The aim of this study is to investigate a redox behavior of Fe(II)/Fe(III) in the presence of selected amino acids (Fig. 1) using cyclic voltammetry in order to assess a degree of risk that the agent will interact with available iron sources in the human body including ferritin in the human brain.

2. Material and methods

2.1 Chemical and Reagents

All chemicals used in this work were of analytical grade (Merck or Sigma Aldrich). Stock solution of 0.01 M phosphate buffer saline (pH = 7.4) was used as a supporting electrolyte. Before use it was diluted appropriately.

2.2 Apparatus

All measurements were performed with a computer-controlled electrochemical analyzer (model PGSTAT 302N, Metrohm Autolab) operated *via* the NOVA 1.9 software (Metrohm Autolab B.V.). A conventional three-electrode system was used with modified carbon paste electrode as a working electrode, Ag/AgCl reference electrode and a platinum wire counter electrode. Digital pH meter model inoLab pH 720 was applied for the preparation of the buffer solutions.

2.2 Fabrication of Modified Electrode

The unmodified carbon-paste mixture was prepared by mixing graphite powder with an appropriate amount of paraffin oil (mass ratio 65 : 35). The modified electrode was prepared by mixing unmodified composite with ferrocene (modifier mass fraction, w(Fc) = 0.8 %) and then homogenized by spatula. The resultant modified carbon paste was packed into a piston-driven electrode holder. The electrode surface was renewed by smoothing on wet filter paper before starting a new set of experiments.

3. Results and discussion

Electrochemical behavior of Fe(II)/Fe(III) in the presence of selected amino acids in phosphate buffer saline was studied by cyclic voltammetry. Since the redox couple Fe(II)/Fe(III) is insoluble in aqueous solutions at physiological pH, ferrocene was used as a source of iron which was add to the carbon paste material as a modifier.

Fig. 2. shows the measured cyclic voltamograms of the Fe(II)/Fe(III) redox couple in the absence and presence of the 1 mM amino acids in a phosphate buffer saline solution (pH = 7.4) at a ferrocene (w(Fc) = 0.8 %) modified CPE between -0.3 to 1.1 V (-0.3 to 1.3 V for Trp and Tyr) at scan rate 50 mV s^{-1}.

In the absence of amino acids the oxidation and reduction peak potentials at the ferrocene-modified CPE occurred at 0.356 and 0.231 V, respectively. Under the identical conditions, the behavior of Fe(II)/Fe(III) redox couple was changed after addition of a selected aminoacid. The positive shift of the oxidation peak potential was observed in the case of all amino acids; the most significant differences were found for cysteine (0.388 V), tyrosine (0.419 V) and tryptophan (0.521 V). The negative shift of the reduction peak potential was observed almost in all cases except tryptophan (0.258 V). Similar result were obtained by studying interactions of redox activity of the redox couple Fe(II)/Fe(III) in the presence of nicotine (BRIDGEA *et al.*, 2004).

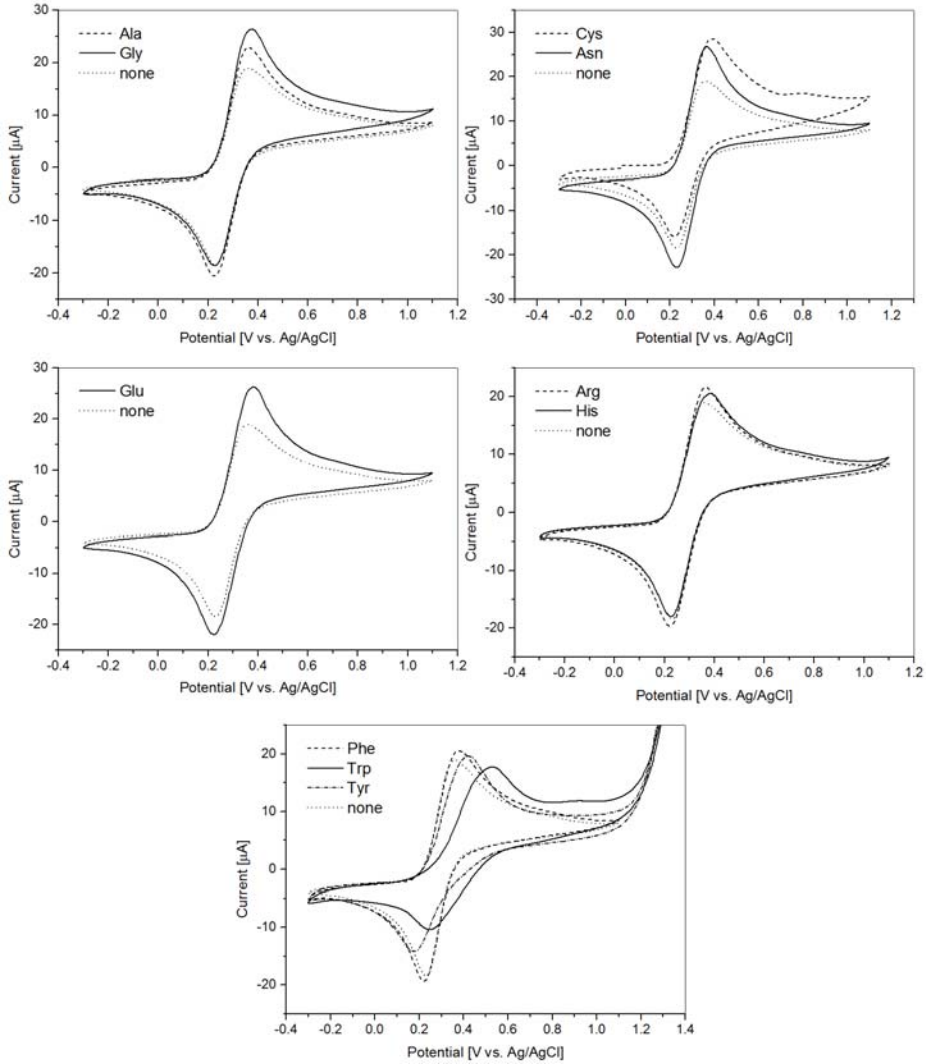

Fig. 2. Cyclic voltammograms of the Fe(II)/Fe(III) redox couple in the absence (dotted) and presence of the 1 mM amino acids in phosphate buffer saline (pH = 7.4) at a ferrocene-modified CPE (w(Fc) = 0.8 %) at the scan rate 50 mV s^{-1}. The amino acids were classified according to their chemical properties mentioned in Fig. 1.

Before the addition of amino acids the oxidation peak current for the ferrocene-modified-CPE was 20.09 μA and the reduction peak was 20.40 μA. After the addition of amino acids a current change was observed. The most common sign of this change war the increasing intensity of the current unlike the study when aqueous Fe(II)/Fe(III) was used (BRIDGEA *et al*., 2004). The oxidation peak current increased almost with all additions of the amino acids, specifically for Ala (24.24 μA), Arg (22.89 μA), Asn

(28.15 μA), Cys (28.08 μA), Glu (26.62 μA), Gly (27.23 μA), His (21.52 μA), Phe (21.77 μA) and Tyr (20.69 μA). However, a decrease of the current to the value of 17.92 μA, was observed for tryptophan along with the potential shifts. The reduction peak current increased with addition of Ala (22.78 μA), Arg (21.92 μA), Asn (25.99 μA), Glu (24.47 μA), Gly (21.35 μA) and Phe (21.32 μA) and decreased with addition of Cys (18.97 μA), His (20.27 μA), Trp (10.44 μA) and Tyr (14.82 μA). Again tryptophan influences this particular electrochemical parameter most significantly.

In an ideal case the ratio of I_{pa}/I_{pc} approaches unity. As it can be seen from Table 1, a value close to unity was obtained only in the absence of amino acids and with the addition of Ala, Arg, Asn, Glu, His, and Phe. This indicates that the presence of the rest of the amino acids (Cys, Gly, Trp, and Tyr) modifies the electrochemical behavior of the redox couple from a reversible diffusion controlled reaction to a kinetically controlled reaction (BARD and FAULKNER, 2001).

The value of ΔE_p varied upon addition of amino acids, with the largest deviation being observed in the case of tyrosine and tryptophan. The value of peak to peak separation was not approximately 0.059 V, which indicates a reversible process but according to the measured ΔE_p our systems exhibited quasi-reversible behavior.

Table 1. Electrochemical characteristics of the Fe(II)/Fe(III) redox couple in the presence and absence of selected amino acids, experimental conditions as in Fig. 2.

Amino Acid	E_{pa} [V]	E_{pc} [V]	I_{pa}/I_{pc}	ΔE_p [V]	$E_{1/2}$ [V]
none	0.356	0.231	0.985	0.125	0.294
Ala	0.359	0.227	1.064	0.132	0.293
Arg	0.364	0.224	1.044	0.134	0.296
Asn	0.363	0.229	1.083	0.168	0.304
Cys	0.388	0.219	1.480	0.168	0.304
Glu	0.360	0.226	1.088	0.134	0.293
Gly	0.375	0.228	1.275	0.147	0.301
His	0.380	0.229	1.062	0.151	0.304
Phe	0.373	0.222	1.021	0.151	0.297
Trp	0.521	0.258	1.717	0.264	0.390
Tyr	0.419	0.177	1.395	0.242	0.298

4. Conclusions

Cyclic voltammetry experiments revealed an alteration in the electrochemical behavior of the Fe(II)/Fe(III) redox couple in the presence of selected amino acids. The oxidation and reduction potentials both shifted, generally becoming more positive and negative, respectively, while the current intensity increased or decreased. A value

for I_{pa}/I_{pc} close to unity was obtained only in the absence of amino acids and with the addition of Ala, Arg, Asn, Glu, His and Phe. The value of ΔE_p varied upon addition of amino acids indicating that our systems exhibited quasi-reversible behavior. Cyclic voltammetry experiments revealed the capability of amino acids to change the electrochemical behavior of the Fe(II)/Fe(III) redox couple.

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