Electrochimica Acta 139 (2014) 29–35

Contents lists available at ScienceDirect

Electrochimica Acta

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

Speciation of biochemically important iron complexes with amino acids: L-aspartic acid and L-aspartic acid - glycine mixture

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ARTICLE INFO

Article history:
Received 23 April 2014
Received in revised form 2 July 2014
Accepted 4 July 2014
Available online 12 July 2014

Keywords:
Iron
L-aspartic acid
Iron-aspartate complex
Stability constants
Mixed ligand complexes

ABSTRACT

Speciation of iron(III) (c_{Fe} = (0.5 - 40) × 10^{-5} mol dm^{-3}) and indirectly of iron(II), in aqueous solution of L-aspartic acid and aspartic acid - glycine mixture was investigated by square-wave voltammetry on a static mercury drop electrode. A reversible, one-electron reduction process of iron(III)-aspartate complexes with peak potential between +0.04 and -0.17 V was recorded, depending on the solution pH (4.5–8.6) and concentration of aspartic acid (c_{Asp} = 0.01 - 0.4 mol dm^{-3}). Cumulative stability constants of iron(III)-aspartate complexes: $FeAsp^+$ (log K = 13.16 ± 0.01), $FeAspOH^0$ (log β = 20.76 ± 0.02), $FeAsp(OH)_2^-$ (log β = 27.77 ± 0.12) and $Fe(Asp)_2^-$ (log β = 17.62 ± 0.10); and iron(II)-aspartate complexes: $FeAsp^0$ (log K = 4.17 ± 0.11) and $Fe(Asp)_2^{2-}$ (log β = 6.53 ± 0.11), were determined at I_c = 0.5 mol dm^{-3} and 25 ± 1 °C. In the solution with mixture of amino acids, aspartic acid and glycine, a formation of mixed (Fe(III)-aspartate-glycine) complexes occurred. The reduction process was also shown to be reversible, so stability constants of mixed complexes (iron(III): $FeAspGly^0$ (log β = 17.35 ± 0.02), $FeAspGly(OH)^-$ (log β = 23.87 ± 0.33), and iron(II): $FeAspGly^-$ (log β = 6.83 ± 0.23)) were calculated, as well.

1. Introduction

Iron is one of the most important trace metals in biochemistry, since it is essential element for virtually every living cell. Thus it occurs in humans as both, iron(III) and iron(II). The biochemistry of iron, regardless of its oxidation state, is controlled by primary coordination sphere that usually involves different naturally occurring ligands (*e.g.* porphyrins, organic acids, amino acids, *etc.*). Therefore, a study of iron complexation with amino acids is relevant for understanding its interactions with expected biological ligands [1–3]. Moreover, in respect to the iron requirements in humans, iron(II) complexes with amino acids are used as a supplement for anemic persons [4–6]. These complexes justify several criteria [5] that define one form of iron as nutritionally functional: low molecular weight of the complex, nutritionally functional stability constant of the complex and the ligand should be metabolized by the body [5].

Amino acids are biologically important organic compounds that, besides their basic function as being building blocks of proteins, perform other critical roles in processes such as neurotransmitter transport and biosynthesis. Being produced as exudate and decomposition product, amino acids are present in terrestrial and aquatic environments, too. They are important part of a dissolved organic matter (DOM) with significant speciation capacity for trace metals in DOM abundant natural waters (*e.g.* coastal waters, estuarine waters and interstitial pore waters) [7]. Since living cells and other natural environments (aquatic or terrestrial environment), contain different amino acids with diverse avidity toward metal ion [1] it is important to elucidate each amino acid's capability to form mixed ligand complexes with metal ion. Namely, in biological systems metal ions usually combine with two different ligands [8] and mixed ligand complexes play crucial role in the catalytic centers of metalloenzymes. Investigations of interaction between transition metals and various amino acids and their ternary complexes can thus be used as metalloenzyme models [9,10]. Therefore, for determination and understanding of metal (iron) speciation in different natural environments, as well as for metalloenzyme models, the essential is the knowledge on stability constants of all possible metal complexes that could be formed in mentioned natural environment [11]. Speciation of metal will finally define its solubility, reactivity, bioavailability and further toxicity [11,12].

In this work, voltammetry was applied for the first time for the investigation of iron speciation in aqueous solution of aspartic acid, as well as for the examination of iron mixed ligand complex with aspartic acid and glycine. Only few papers dealt with iron-aspartate complexes in aqueous solutions [13–17] leaving the characterisation of iron-aspartate system incomplete. Taking into

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http://dx.doi.org/10.1016/j.electacta.2014.07.006
0013-4686/

account that iron in both oxidation states, +3 and +2, has six coordination sites, and that aspartic acid is three-dentate ligand [14], it can be presumed that iron(III) would form complexes with two aspartate molecules, as well as mixed aspartate-hydroxide complexes as Dmitrieva et al. [17] qualitatively described but stability constant values are up to date unknown. Up to our knowledge, stability constants of mixed iron-aspartate-glycine complexes are also unknown. Voltammetric technique is specially suitable since it enable i) measurement of low concentrations of iron(III) (< 10^{-5} mol dm^{-3}) and ii) determination of stability constant. This work represents our extended investigations on the iron(III) speciation in aqueous solution of biologically important organic ligands [18–21].

2. Experimental

2.1. Equipment and measurements procedure

Experiments were performed using a μ-AUTOLAB multi-mode potentiostat (ECO Chemie, Utrecht, The Netherlands) equipped with a Metrohm 663 VA stand (Metrohm, Herisau, Switzerland). GPES 4.9 software was used for the instrument computer-control, while voltammograms were analyzed by using the *E*lectro*C*hemical *D*ata *SOFT*ware (ECDSOFT) program that was made in our laboratory [22]. The working electrode was a static mercury drop electrode (SMDE, size 2, *i.e.* 0.40 mm^2), the counter electrode was a glassy carbon rode and the reference electrode was Ag/AgCl(sat. NaCl) (+0.197 V *vs.* standard hydrogen electrode, SHE). Measurements were performed in an electroanalytical quartz cell at (25 ± 1) °C. A square-wave (SW) voltammetry technique with cathodic scan direction was applied, with the optimal SW-parameters: pulse amplitude, a = 25 mV; frequency, f = 50 s^{-1}; potential step increment, E_{inc} = 2 mV. Prior to electrochemical measurements, the solutions were deaerated by bubbling with extra pure nitrogen for about 15 min with stirring (3000 turns per minute). During measurements nitrogen circulated above the solution.

The pHs of the solutions were measured with a combined glass-Ag/AgCl electrode connected to an ATI Orion PerpHecTMeter, model 320 (Cambridge, USA). pH electrode was immersed into electroanalytical cell throughout slit on the cells top cover, thus providing a measurement of the solution's pH before, during and after the voltammetric scan. Calibration of the glass electrode was performed using standard buffer solutions.

2.2. Chemicals and solutions

Standard solution of iron(III) nitrate (1.79×10^{-2} mol dm^{-3}) (Fluka Chemie, Buchs, Switzerland) was used in all experiments. The aqueous stock solution of L - aspartic acid (*p.a.*, Merck, Darmstadt, Germany) was prepared by dissolving the solid in deionized water from a Milli-pore Mili-Q system (Bedford, USA) with addition of *p.a.* NaOH (Merck) to enhance its solubility. Glycine (*p.a.*, Merck, Darmstadt, Germany) and sodium perchlorate ($NaClO_4 \times H_2O$, *p.a.*, Fluka Chemie, Buchs, Switzerland) were also dissolved in deionized water to obtain stock aqueous solution.

The pH of solutions was adjusted by adding diluted *s.p.* $HClO_4$ or *p.a.* NaOH.

3. Results and discussion

3.1. Speciation of iron in solution of L-aspartic acid

Aspartic acid (Asp) with a general formula $HOOCCH(NH_3^+)CH_2COOH$ (fully protonated form, H_3Asp^+), is a non-essential α-amino acid and one of the 20 "standard" amino acids commonly found in animal proteins [23]. L-aspartic acid plays an important role in active sites of certain enzymes, for example in aspartic proteases that contain even two molecules of aspartic acid in the active site [24].

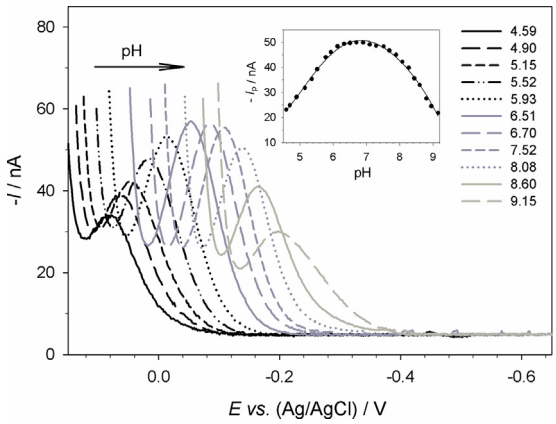

Fig. 1. SW voltammograms of Fe(III)-aspartate complexes in 0.5 mol L^{-1} $NaClO_4$ at different pH. $c_{Fe} = 2 \times 10^{-5}$ mol dm^{-3}; c_{Asp} = 0.4 mol dm^{-3}. Inset: dependence of reduction peak current on pH.

3.1.1. Electrochemical measurements

Voltammograms recorded in the acidic aqueous solution of L-aspartic acid showed three SW - reduction peaks with the peak potentials between -0.75 V and -1.15 V (pH = 2.0–5.5). This voltammetric peaks were irreversible and most probably related with the reduction of proton from solution catalyzed by the oxygen and nitrogen atoms from the aspartic acid [25].

With the total iron(III) concentration $c_{Fe} = 2 \times 10^{-5}$ mol dm^{-3} and total aspartic acid concentration c_{Asp} = 0.4 mol dm^{-3} in the pH range between pH 4 and 9, reduction of iron(III)-aspartate complexes was registered as shown in Fig. 1. As can be noticed in inset of the Fig. 1, the highest reduction currents were recorded between pH of about 6.2 and 7.6. The increase of the peak current until pH 6.2 was related with gradual enhancement of the Fe(III)-Asp complex(es) concentration because of increase of a fully deprotonated aspartic acid form that bounds Fe^{3+} ion. Decrease of the peak current above 7.6 indicated gradual formation of various (in)soluble iron(III)-hydrolyzed species. The current shift in the left part of "detection window" toward more negative potentials (Fig. 1) was most probably related with pH-influence on reduction of mercury-aspartate complex(es) formed at the electrode surface. Under the conditions of applied technique the process of iron(III)-aspartate complex(es) reduction was shown to be reversible, therefore, stability constants of iron-aspartate complexes were calculated from the E_p *vs.* pH dependence as described in the next section (Section 3.2).

The lowest concentration with which iron(III)-aspartate reduction peak was recorded was c_{Asp} = 0.01 mol dm^{-3} (pH = 7.0, $c_{Fe} = 2 \times 10^{-5}$ mol dm^{-3}, I_c = 0.5 mol dm^{-3} ($NaClO_4$)), Fig. 2. However, successive voltammograms with this total aspartate concentration showed the kinetics of the iron(III)-aspartate exchange with $[Fe(OH)_n]^{3-n}$ complexes and therefore, dissociation of iron(III)-aspartate complex that reflected as gradual decrease of iron(III)-aspartate reduction peak at -0.04 V, Fig. 2. With 0.01 mol dm^{-3} of aspartate, the reduction peak was recorded only 1.2 hours from the addition of iron(III) to the solution (Fig. 2). Regarding the hydrolysis of iron(III)-amino acids complexes, Cuculić *et al.* [26,27] found similar behavior in the iron(III)-glycine system. Voltammetric peak at -0.35 V (Fig. 2) was characterized as one-electron irreversible reduction with reactant adsorption of the $[Fe(OH)_n]^{3-n}$ species [27]. Other reduction peaks (Fig. 2) at -1.42 V and -1.56 V