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Nanostructured electrochemical detector for the quantification of amino acids related to metabolic diseases

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ABSTRACT

L-Arginine, L-ornithine and L-citrulline are amino acids intermediates in many metabolic pathways such as urea cycle. The concentration of these amino acids in urine or blood is indicative of proper urea degradation. An inappropriate degradation has great implications in various metabolic disorders. In particular, the concentration of L-arginine and L-citrulline builds up in blood when Argininemia or Citrullinemia disorders are diagnosed. Hence, their fast and reproducible determination plays a key role in metabolic studies and suitable analytical methods are necessary for their quantitative analysis in biological samples. In this study, a simple analytical approach for the determination of L-arginine, L-ornithine and L-citrulline in urine and serum by anion-exchange chromatography, without derivatization schemes is described. A carbon screen-printed electrode containing single wall carbon nanotubes modified with highly-dense electrogenerated $Ni(OH)_2$ nanoparticles is used as liquid chromatography electrochemical detector. At a first step, micro structures of a novel paddle-wheel tetrakis-acetate di-nickel(II) complex $[Ni_2(CH_3COO)_4]$ were electrosynthetized on a gold coil from its precursor tetrakis-monothioacetate di-nickel paddle-wheel complex $[Ni_2(CH_3COS)_4$ EtOH]. These microstructures serve the pattern to produce highly dense and uniform coverage of small size $Ni(OH)_2$ nanoparticles (3 nm) on the electrode surface by a simple procedure consisting of successive cyclic potential scans in alkaline medium. The resulting modified electrode presents a potent electrocatalytic activity towards the oxidation of L-arginine, L-ornithine and L-citrulline allowing, coupled to a chromatographic system, quantify these amino acids directly in biological samples.

1. Introduction

The urea cycle consists on a cycle of six metabolic reactions that transforms the ammonia from the amino acids degradation deposited in the hepatocytes to urea, which is excreted by the kidneys. During the cycle, arginine is cleaved into urea and ornithine. In a next step, ornithine and carbamoyl phosphate form L-citrulline that is coupled with aspartate to form argininosuccinate. Finally, argininosuccinate is cleaved into arginine and fumarate, closing the urea cycle [1,2]. Different enzymes are needed to carry out the above reactions. As the properly urea biosynthesis is necessary, a deficiency of one of the six involved enzymes causes urea cycle disorders, which belong to the generic group of the amino acid disorders. Inherited or acquired disorders in the urea cycle can lead to severe clinical conditions like hyperammonemia [3]. The biochemical markers of these disorders are ammonium and a pattern of amino acids in plasma and urine. Hence, to characterize the status of the urea cycle and its implications in various clinical conditions, the concentrations of arginine and citrulline have recently become the focus of some scientific research [4,5]. In particular, it is described that the concentration of these two amino acids builds up in blood when Argininemia or Citrullinemia disorders are diagnosed. On the other hand, arginine and ornithine are also involved in other amino acid disorder, like Cystinuria. In this case, patients show moderately increased excretion of these two amino acids [6,7]. Therefore, due to the many important biological functions, arginine, ornithine and citrulline analysis plays a key role in metabolic studies and suitable, fast and reproducible, analytical methods for their determination

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in biological samples are needed. The determination of natural amino acids and their related compounds is not always simple, because these compounds lack of natural functional groups capable to be detected in a direct form by photometric and fluorimetric methods. Indeed, analytical methods employed for the quantification of free amino acid in biological samples include CE, GC or LC after chemical pre- or post- column derivatization followed by UV, fluorescent or MS detection of the amino acid derivative [7–13]. However, these methods are often time-consuming due to tedious derivatization procedures and long running time. Moreover, derivatives are not always stable. Thus, a direct detection method that does not require derivatization processes is preferred, when available, for convenience, rapidity and simplicity. In this sense, electrochemical sensors can be a good alternative because they offer some advantages such as high sensitivity, rapid response, low cost, miniaturization and more importantly, the possibility to construct portable devices easy to use for point of care. Recently, non-enzymatic electrochemical sensors, in particular, those based on several transition metal-based electrodes such as copper [14], nickel-titanium [15], nickel-gold [16] as well as chemically modified carbon or metal electrodes [17–20] have been employed as electrocatalytic materials in electrochemical sensors for the direct determination of underivatized amino acids. Among them, nickel hydroxide has been the subject of much investigation. The electrocatalytic effect is believe to arise from unpaired d electrons or empty d orbitals associated with the oxidized form of Ni (NiOOH; i.e., Ni^{3+}), which is available for bond formation with adsorbed or in solution species [21–23].

On the other hand, it has been demonstrated that just moving from the bulk material to nanosized structure can often significantly increase the electrocatalytic activity of some materials. Thus, decorating substrates with isolated nanoparticles or nanostructures is a frequently way to prepare efficient electrocatalyst. In this sense, nickel-carbon nanotubes [24], copper [25], NiO [26], and Co nanoparticles [27], as well as Fe_3O_4-graphene oxide nanocomposites [28], nanoporous nickel/boron-doped diamond films [29] and nickel nanosheet/graphene composite [30] have been used for the catalytic oxidation of amino acids. However, as far as we know, there are few cases in which electrodes modified with these nanostructures have been used as electrochemical detectors in chromatographic systems for amino acids determination [25]. Moreover their application to the quantification of amino acids related to metabolic diseases in biological samples, such as human urine or serum, has not been described yet.

Recently, we reported on the development of $Ni(OH)_2$ nanoparticles based platforms, electrogenerated from a dinuclear paddle-wheel nickel complex (tetrakis-monothioacetate di-nickel(II)), with a potent electrocatalytic activity towards the electro-oxidation of carbohydrates and insulin in alkaline medium [31,32]. In the present work, using this platform as amperometric detector in anion-exchange high performance liquid chromatography (HPLC), we describe the development of a simple analytical approach for the determination of underivatized amino acids. The applicability of the developed methodology to the direct determination of metabolic diseases related amino acids in biological samples is demonstrated.

2. Experimental

2.1. Reagents

Tetrakis-monothioacetate dinickel paddle-wheel complex, $[Ni_2(CH_3COS)_4$ EtOH], was prepared as previously described [33] with slight modifications. Briefly, a warm ethanolic solution of thioacetic acid (0.037 mol) was treated with [$NiCO_3$ $2Ni(OH)_2$ $4H_2O$] (0.018 mol) in a portion-wise fashion. Color changed from light orange to deep red in about 30 min and the mixture was stirred further for 4 h. The suspension formed was filtered and the precipitated discarded, filtrate was allowed to stand overnight at −10 °C to yield deep red plate-like crystals, 1.854 g (43%). In order to characterize the compound, RMN and Elemental Analysis were carried out as previously reported [31]. L-Arginine, L-ornithine, L-citrulline, human serum, perchloric acid, acetonitrile, chloroform, dimethylformamide and sodium hydroxide were purchased from Sigma Aldrich. Stock solutions containing 0.5 mM of each amino acid were freshly prepared daily in 0.1 M NaOH aqueous solution.

2.2. Apparatus

All electrochemical measurements were carried out using an Autolab potentiostat/galvanostat type PGSTAT 302N (Eco Chemie, Netherlands) using the software package GPES 4.9. Integrated single walled carbon nanotubes modified screen printed electrodes (SWCNTSPEs) from Dropsens were used. They include a silver pseudoreference electrode and a carbon counter electrode. For the electrosynthesis of $[Ni_2(CH_3COO)_4]$ a gold wire coil (Goodfellow) was used as working electrode, a platinum wire as counter electrode and a special Calomel electrode for organic medium from Radiometer Analytical as reference electrode. A wall jet flow cell from Dropsens (model FLWCL) was employed for on-line analysis. An Ismatec peristaltic pump was used to draw solution continuously through the cell. A low-pressure rotary injection valve with an exchangeable sample loop was used in the flow system. The supporting electrolyte was 0.1 M NaOH.

The HPLC system consisted of a PU-2080 Plus Pump from Jasco equipped with a vacuum degasser and an injector with a 20 μl sample loop (Rheodyne). Separations were carried out using a Dionex AminoPac PA10 anion-exchange analytical column (4 × 250 mm) from Thermo Fisher. The HPLC system was operated in isocratic mode at 0.75 ml min^{-1} flow rate. The mobile phase consisted of 60 mM NaOH plus 3% acetonitrile as organic modifier and was blanketed by inert gas and passed through the on-line degasser in the HPLC pump. For amperometric detection, an Autolab equipped with the Dropsens wall jet cell was used.

For the nanoscale catalysts characterization by transmission electron microscopy (TEM), a carbon film covered copper grids were used and images were recorded with a JEOL JEM 2100 electron microscope.

2.3. Procedures

2.3.1. Preparation of $Ni(OH)_2$NPs/SWCNT modified screen printed electrode

$Ni(OH)_2$ nanoparticles were generated along the SWCNTSPE according to the following procedure: the tetrakis-acetate di-nickel paddle-wheel complex $[Ni_2(CH_3COO)_4]$ was electrosynthetized onto a gold wire coil from a solution containing 0.25 mM of the di-nickel paddle-wheel complex $[Ni_2(CH_3COS)_4$ EtOH] in 0.1 M TBAP/$CHCl_3$ by applying 1.15 V during 10,000 s. The formation of $[Ni_2(CH_3COO)_4]$ is corroborated by the colorless of the solution. The electrosynthetized material remains onto the gold wire coil since it is not soluble in chloroform. Then, the wire coil was rinsed with chloroform and the $[Ni_2(CH_3COO)_4]$ was removed from it with acetonitrile. SWCNTSPE were modified by transferring 5 μl of the electrosynthetized $[Ni_2(CH_3COO)_4]$ solution in acetonitrile onto its surface followed by air-drying at room temperature. Finally, 50 potential cycles between +0.2 and +0.7 V at 0.1 V s^{-1} in 0.1 M NaOH solution were applied to obtain $Ni(OH)_2$NPs/SWCNTSPE.

For TEM measurements, a copper grid was modified with a 1 mg ml^{-1} dispersion of single walled carbon nanotubes (Sigma Aldrich) in dimethylformamide and allowed to dry in air at room