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Electrochemical and spectral study on the effects of Al(III) and nano-Al_{13} species on glutamate dehydrogenase activity

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ABSTRACT

A functionalized multi-wall nanotube (MWNT) modified glass carbon electrode (GCE) was used to study the effects of aluminum species on glutamate dehydrogenase (GLDH) activity by monitoring amperometric *i–t* curve for the oxidation of the enzymatically generated NADH. The conformational changes of the coenzyme nicotinamide adenine dinucleotide (NAD^+) induced by Al(III) and nanometer-sized tridecameric aluminum polycation (nano-Al_{13}) were investigated by the fluorescence technique. The results showed that the electrochemical method may be a potential tool to investigate the activity of enzymes in the biological system. It may also be useful in studying the effects of nano-sized aluminum compounds on biomolecules in order to discuss their safety to the environment and human.

1. Introduction

Aluminum has been recognized as a neurotoxic agent in animals for more than 100 years because it has been shown interfering with many key enzymatic processes of brain that basically control cell functions. It is also known that aluminum is an important relevant etiological factor in several diseases, such as dialysis treatment, Alzheimer's disease, Parkinson's disease, osteomalacia and anemia [1–5]. Moreover, it has been proposed that various species of a metal may have different toxicity and behavior to enzymes and other biomolecules [6]. Therefore, it is reasonable to assume that the various species of Al may display different effects on the activity of enzymes.

Nanoparticles are a novel material with a vast array of exotic electronic, catalytic, and chemical properties created today by nanoscientists, and have been widely used in diverse fields. In recent years, the potential effects of nanoparticles which lead to unforeseen health or environmental hazards to humans or other species have raised considerable concerns [7]. The toxicity of some nanomaterials, such as fullerene and its derivatives, quantum dots, and nano-oxide (titanium dioxide, silica, zinc oxide, alumina, etc.) has been under investigation [8–12]. Recently, researchers have paid their attention to the toxicity of nanometer-sized tridecameric aluminum polycation (nano-Al_{13}, also called nanopolynuclear Al_{13}) towards the activity of proteins and enzymes at the molecular level. On one hand, the nano-Al_{13} is wildly used in water treatment because of its effective coagulation effects and rapid aggregation velocity in a relatively wide range of pH. On the other hand, the nano-Al_{13} is more likely to the real species under the physiological condition, and the process of $Al(OH)_3$ formation required the presence of nanopolynuclear Al_{13} as a precursor. Moreover, Valeswara Rao et al. [13] have provided the evidence for the presence of the Al_{13} polymer inside the synaptosomes. Therefore, it is of significant value to investigate the toxicity of Al_{13}.

L-Glutamate dehydrogenase (GLDH) is a key enzymatic link between catabolic and biosynthetic pathways and it has been found in both higher and lower organisms [14]. This enzyme may be of particular importance not only in the plant biology, but also in the animal nerve system since the pathology of some disorders associated with GLDH deficiency is restricted to brain. It has been found that the enzyme can provide a pivotal link between carbohydrate and nitrogen metabolism [15,16]. In the oxidative deamination reaction, GLDH feeds the tricarboxylic acid cycle (TCA) by converting L-glutamate (L-Glu) to α-ketoglutarate (α-KG) with using the nicotinamide adenine dinucleotide (NAD^+) as coenzyme. The inhibitory effects of aluminum occur in the formation of α-KG in the oxidative deamination reaction process, which may influence

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the amino acid synthesis [17]. In the previous work, Yang et al. [18] reported that aluminum promotes the tautomerization of α-KG, a substrate of GLDH, to its enolic form in the aqueous solutions. Bi et al. [19] showed that the toxic effect of nano-Al_{13} on the activity of lactate dehydrogenase (LDH) is much higher than that of the free Al(III), and the critical concentration of nano-Al_{13} was found at least 2-fold higher than that of Al(III) at the maximal inhibition place from the molecular level.

It remains a challenge to investigate how an enzyme alters its structure and properties in the presence of metal ions and nanoparticles because most enzymes consist of several subunits with a very large molecular mass. Electrochemical analysis has been considered as a powerful tool to detect metal ions and biomolecules in the biological system with a number of remarkable advantages such as high sensitivity, fast and reliable results, simple instrumentation and operation procedure and low cost. Electrochemical methods have been used in enzyme studies in the past few decades. Gao and Xin [20] studied the effects of lanthanide ions on the kinetics of GLDH by a chronoamperometric method using the bare glassy carbon electrode (GCE). Bi et al. [21] investigated the effect of Al(III) and Al_{13} on the LDH activity by differential pulse voltammetry on the hanging mercury drop electrode (HMDE). Yang et al. [22] reported the electrochemical studies of the inhibition and activation effects of Al(III) on the activity of bovine liver GLDH with HMDE. In recent years, more and more researchers have turned their attention to the application of modified electrodes on biochemistry for their excellent catalytic properties. Zhuang et al. [23] explored the electrochemical properties of single-walled carbon nanotubes (SWNT) which were not functionalized as nanometer-sized activators in enzyme-catalyzed reaction and the experiment results showed that the modified electrode could be successfully used to monitor the activity of LDH.

In this work, a functionalized multi-walled nanotube (MWNT) modified GCE was used to detect the oxidation current of NADH which were generated in the enzymatic reaction, and the current can be used as a measure for the GLDH activity. Based on the sensitive and stable *i*–*t* curve of NADH on the modified electrode, we successfully showed the effects of various species of aluminum on the activity of GLDH, especially the nano-Al_{13}. Meanwhile, some other factors which may influence GLDH activities were investigated. Our experiments showed that the proposed method can be employed to living systems avoiding the toxicity of the mercury electrode. Furthermore, the fluorescence technique was used to study the conformational changes of the coenzyme NAD^+, which may be induced by Al(III) or nano-Al_{13} species in aqueous solution. Other spectral techniques, including ^{27}Al NMR and XRD, were used to characterize the nano-Al_{13} species.

2. Experimental

2.1. Materials and instrumentation

L-Glutamate dehydrogenase (GLDH, EC, 1.3.1.4) from bovine liver, L-glutamate, β-nicotinamide adenine dinucleotide (NADH, NAD^+) were purchased from Sigma Co. (St. Louis, MO, USA). Multi-walled carbon nanotube (MWNT, <10 nm in diameter and 0.5–500 nm in length with the purity of more than 95) was obtained from Shenzhen Nanotech Port Co., Ltd., Shenzhen. All other chemicals were of analytical regent grades. All solutions were prepared with doubly distilled water. Tris–HCl buffer solution was prepared by dissolving appropriate amount of Tris aminomethane (hydroxymethyl), and then adjusting pH by concentrated hydrochloric acid to various values. The Al stock solution was prepared by dissolving high purity metallic Al powder (99.99%) in hydrochloric acid at pH < 2 to prevent hydrolysis of Al^{3+} ion. Then the stock solution was diluted by doubly distilled water.

Electrochemical measurements were performed by using CHI660B (CH Instruments, Chenhua Inc., Shanghai, China). A three-electrode configuration was employed which consist of a MWNT-modified GC or a bare GC electrode as a working electrode, platinum wire and saturated calomel electrode (SCE) as auxiliary electrode and reference electrode, respectively. Before the electrochemical experiments, the Tris–HCl buffer solution was degassed for at least 20 min by bubbling high-purity nitrogen gas and the nitrogen environment was then kept over solution throughout the measurement. Fourier transform infrared (ATR-FTIR) of spectrum of MWNTs-GCE was recorded on a Cary 5000 spectrophotometer (Varian Co., USA). Fluorescence spectrum was measured on a LS-50B of spectrofluorimeter (PerkinElmer Co., USA). X-ray diffraction (XRD) pattern was obtained on a X'TRA diffractometer (ARL Co., Switzerland). The instrumental setting was Cu Kα radiation. Data were collected from 2θ degree of 5–60° with a scanning rate of 1° 2θ min^{-1}. ^{27}Al NMR (DRX 500, Bruker. Co., Switzerland) spectrum was utilized to certify the existence of Al_{13} Keggin structure.

2.2. Preparation of functionalized MWNT

Twenty milligrams of MWNT was dispersed in 30 mL 30% HNO_3, and the resultant mixture was then refluxed for 24 h at 140 °C as given in Ref. [24]. The resulting suspension was centrifuged and the precipitate was washed with water to obtain carboxylic group functionalized MWNT. The obtained functionalized MWNT was completely dispersed in water at pH 1.0 for perpetrating a 5 mg/mL MWNT suspension, which was then neutralized to pH 7.0 with 0.1 mol/L NaOH.

2.3. Preparation of MWNT-CHIT suspension

A 1.0 wt% CHIT stock solution was prepared by dissolving chitosan flakes in hot (80–90 °C) aqueous solution of 1% HAc. The solution was cooled to room temperature, and its pH was adjusted to 3.5–5.0 by using concentrated NaOH solution. A MWNT-CHIT solution was prepared by mixing the obtained functionalized MWNT suspension and CHIT solution. The aqueous mixture was then sonicated for 30 min to obtain a homogeneous suspension.

2.4. Preparation of MWNT-CHIT modified electrode

The glassy carbon electrode (GCE) was successively polished to a mirror finish using 0.3-μm and 0.5-μm alumina slurry followed by rinsing thoroughly with doubly distilled water. After successive sonication in absolute alcohol and doubly distilled water, the electrode was rinsed with doubly distilled water and allowed to dry at room temperature. The MWNT-CHIT modified electrode was prepared by casting 1.2 μL of MWNT-CHIT solution on the surface of a GCE, and then was dried at room temperature. The electrode was stored in the desiccator when not used.

2.5. Synthesis of nano-polynuclear aluminum sulfate

25 mL of 0.25 mol/L aluminum chloride solution was heated and kept at 70 °C using a thermostat. And 60 mL of 0.25 mol/L NaOH solution was slowly added under continuous stirring and accurate velocity control and the obtained solution was kept at room temperature for 24 h, then 62.5 mL of 0.1 mol/L Na_2SO_4 solution was added. 10 min after the reaction, the solution was filtrated and aged for 48 h. The crystal obtained from centrifugalization was washed twice with distilled water and 70% ethanol solution, respectively, then air-dried and stored in a desiccator for future use. The XRD and NMR spectra of the obtained crystal were measured as shown in Figs. 1 and 2.