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Selective determination of inosine in the presence of uric acid and hypoxanthine using modified electrode

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ABSTRACT

This article describes the selective determination of inosine (INO) in the presence of important physiological interferents, uric acid (UA) and hypoxanthine (HXN), by differential pulse voltammetry at physiological pH (7.2) using the electropolymerized film of 3-amino-5-mercapto-1,2,4-triazole (p-AMTa) modified glassy carbon (GC) electrode. The electropolymerization of AMTa was carried out by the potentiodynamic method in 0.1 M H_2SO_4. An atomic force microscopy image shows that the p-AMTa film contains a spherical-like structure. Bare GC electrode fails to resolve the voltammetric signal of INO in the presence of UA and HXN due to the surface fouling caused by the oxidized products of UA and HXN. However, p-AMTa film modified GC electrode (p-AMTa electrode) not only separates the voltammetric signals of UA, HXN, and INO, with potential differences of 730 mV between UA and HXN and 310 mV between HXN and INO, but also shows enhanced oxidation current for them. The selective determination of INO in the presence of UA and HXN at physiological pH was achieved for the first time. Using the amperometric method, we achieved the lowest detection of 50 nM for INO. The practical application of the current modified electrode was demonstrated by determining the concentration of INO in human blood serum and urine samples.

Very recently, electropolymerization of heterocyclic compounds such as 2,5-dimercapto-1,3,4-thiadizole [1], 3-amino-1,2,4-triazole [2], 5-vinyltetrazole [3], 5-amino-2-mercapto-1,3,4-thiadiazole (AMT)[1] [4], 2-amino-1,3,4-thiadiazole (ATD) [5], and bis-phenothiazin-3-yl methane [6] were studied. These polymer films showed excellent electrocatalytic activity toward several biomolecules [6–9]. Inosine (INO) is a purine nucleoside that is formed when hypoxanthine (HXN) is attached to a D-ribose ring via β-N9-glycosidic bond and is a metabolite of adenosine triphosphate (ATP) degradation [10]. INO, HXN, and uric acid (UA) are derived from the decomposition of ATP [11,12]. Normally, nucleoside concentrations in biological fluids, perfusates, and incubation media are extremely low and rarely exceed 100 nM [13]. INO participates in tumor necrosis factor, which induces nitric oxide production in cultured Sertoli cells [14,15]. INO and its nucleotides play various important roles in the human system. It was directly associated with multiple sclerosis [16], protects against myocardial damage [17], and has anti-inflammatory and immunomodulatory effects [18,19]. Thus, INO was determined from human blood serum [10,20], blood plasma [20–24], and heart samples [25]. The oxidative process of INO involves two electrons and two protons transferring through an intermediate product process [10]. The oxidations of HXN [11] and UA also involve a two-electron process.

Previously, simultaneous determination of nucleosides, including INO, was carried out by microbe column high-performance liquid chromatography (HPLC) with a diode array detector [26], flow injection analysis system [27], multielectrode enzyme sensor [28], and immobilized enzyme membranes [12]. HPLC methods are very tedious and a more time-consuming process, whereas the reproducibility of enzyme-based methods is poor. In addition, very poor detection limits were observed by these methods. Among the different methods, electrochemical methods have received much interest because they are less expensive, more convenient, more selective, and more sensitive. Hence, the individual determination of INO was studied by electrochemical methods [10,21,29]. Enzyme electrode (coimmobilizing nucleoside phosphorylase and xanthine oxidase on a Nafion-coated platinum disk electrode) [29], $La(OH)_3$ nanowires modified carbon paste electrode [10], and single-walled carbon nanotubes modified pyrolytic graphite electrode [21] were used to determine the concentration of INO. Surprisingly, no report was available in the literature for the determination of INO in the presence of important interferents, UA and HXN, by the electrochemical method. Thus, the goal of the

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[1] *Abbreviations used:* AMT, 5-amino-2-mercapto-1,3,4-thiadiazole; ATD, 2-amino-1,3,4-thiadiazole; INO, inosine; HXN, hypoxanthine; ATP, adenosine triphosphate; UA, uric acid; HPLC, high-performance liquid chromatography; p-AMTa, electropolymerized 3-amino-5-mercapto-1,2,4-triazole; S/N, signal/noise ratio; AA, ascorbic acid; PB, phosphate buffer; AFM, atomic force microscopy; ITO, indium tin oxide; DPV, differential pulse voltammogram.

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current work was to selectively determine INO in the presence of UA and HXN by differential pulse voltammetry using the electropolymerized 3-amino-5-mercapto-1,2,4-triazole (p-AMTa) film. The fabricated p-AMTa electrode was used for the simultaneous and selective determination of INO in the presence of important interferents (UA and HXN). Using the amperometric method, the lowest detection of INO was achieved at 2.97×10^{-10} M (signal/noise ratio [S/N] = 3). The p-AMTa electrode shows selectivity toward INO in the presence of 1000-fold common interferents such as $MgSO_4$, $CaCl_2$, NaCl, K_2CO_3, NaF, NH_4Cl, urea, glucose, oxalate, glycine, and alanine.

Materials and methods

Chemicals

AMTa (95%, CAS no. 16691-43-3), UA (⩾99%, CAS no. 69-93-2), HXN (⩾99%, CAS no. 68-94-0), INO (⩾99%, CAS no. 58-63-9), and ascorbic acid (AA, ⩾99%, CAS no. 50-81-7) were purchased from Aldrich and used as received. All other chemicals used in this investigation were of analytical grade. Phosphate buffer (PB) solution (pH 7.2) was prepared using Na_2HPO_4 (99.5%, CAS no. 10028-24-7) and NaH_2PO_4 (98.0–100.5%, CAS no. 13472-35-0) and were purchased from Merck and used as received. Double distilled water was used to prepare the solutions used in the current investigation.

Instrumentation

Electrochemical measurements were performed in a conventional two-compartment three-electrode cell with a mirror polished 3-mm GC electrode as working electrode, Pt wire as counter electrode, and NaCl saturated Ag/AgCl as reference electrode. All of the electrochemical measurements were carried out with a CHI model 634B electrochemical workstation (CH Instruments, Austin, TX, USA). For DPV measurements, a pulse width of 0.06 s, an amplitude of 0.05 V, a sample period of 0.02 s, and a pulse period of 0.20 s were used. For chronoamperometric measurements, a pulse width of 0.25 s and a potential step of 1 were used. All of the electrochemical measurements were carried out under a nitrogen atmosphere at 27 °C. The tapping mode atomic force microscopy (AFM) images were recorded using a Nanoscope (IV) instrument (Vecco). For AFM measurements, indium tin oxide (ITO) purchased from Asahi Beer Optical (Japan) was used as a substrate.

Fabrication of p-AMTa modified GC electrode

GC electrode was polished with 0.05-μm alumina slurry and then rinsed thoroughly with water. Then the electrode was sonicated in water for 5 min to remove any adsorbed alumina particles. The electropolymerization of AMTa on GC electrode was carried out by 15 successive potential sweeps between −0.20 and +1.70 V at a scan rate of 50 mV s^{-1} in 1 mM AMTa containing 0.1 M H_2SO_4 [30]. We obtained identical electropolymerization behavior for AMTa on ITO surface.

Results and discussion

AFM studies of p-AMTa film

The size and morphology of the p-AMTa film was investigated by AFM. The tapping mode two-dimensional (Fig. 1A) and three-dimensional (Fig. 1B) AFM images of p-AMTa on an ITO plate (2×2 μm) show a spherical-like structure with a thickness of approximately 30 nm. The diameter of each particle was found to be 20–30 nm.

Electrochemical behavior of INO at bare and p-AMTa modified GC electrodes

We examined the electrocatalytic activity of p-AMTa electrode by varying the potential cycles and pH. We found that the p-AMTa film deposited by 15 cycles on GC electrode showed higher electrocatalytic activity toward INO than the films deposited by more than 15 cycles. Furthermore, we performed the oxidation of INO at different pH values using p-AMTa electrode. We obtained higher oxidation current with less positive oxidation potential for INO at pH 7.2. Thus, p-AMTa film deposited by 15 cycles and 0.2 M PB solution (pH 7.2) were chosen for the determination of INO. Fig. 2 shows the differential pulse voltammograms (DPVs) obtained for 0.5 mM INO at bare GC and p-AMTa electrodes in 0.2 M PB solution (pH 7.2).

In Fig. 2, bare GC electrode shows a broad oxidation peak for INO at 1.34 V in the first cycle (curve a). In the subsequent cycles the INO oxidation peak was shifted to a more positive potential, and after eight cycles an ill-defined oxidation wave was obtained (curve b). This is due to a fouling effect caused by the adsorption of oxidized products of INO on the GC electrode surface. The obtained results indicate that bare GC electrode was not suitable for the determination of INO. On the other hand, p-AMTa electrode shows a very sharp oxidation peak at 1.23 V with an enhanced oxidation current for INO in the first cycle (curve c). In the subsequent cycles a stable oxidation peak was observed, and even after eight cycles either no shift in the oxidation peak or a decrease in peak current was observed (curve d). The observed stable oxidation peak indicates that p-AMTa electrode effectively prevents the fouling caused by the oxidized products of INO. Therefore, p-AMTa electrode can be used for the stable determination of INO. Furthermore, p-AMTa electrode shows a 3-fold higher oxidation current for INO compared with bare GC electrode. p-AMTa electrode does not show any redox peak in the absence of INO in 0.2 M PB solution at pH 7.2 (curve e).

We calculated the standard heterogeneous rate constant (k_s) value for INO oxidation at bare and p-AMTa film modified GC electrodes using the Velasco equation [31]:

$$k_s = 1.11 D_o^{1/2} (E_p - E_{p/2})^{-1/2} v^{1/2}, \quad (1)$$

where D_o is the apparent diffusion coefficient, E_p is the oxidation peak potential, $E_{p/2}$ is the half-wave oxidation peak potential, and v is the scan rate. The D_o value was determined using the Cottrell slope obtained by the single potential chronoamperometry technique. D_o values of 4.03×10^{-7} and 2.19×10^{-6} cm^2 s^{-1} were obtained for INO at bare GC and p-AMTa electrodes, respectively. The estimated k_s values for the oxidation of INO at bare GC and p-AMTa electrodes were found to be 5.15×10^{-4} and 1.44×10^{-3} cm s^{-1}, respectively. The higher k_s value obtained for INO at p-AMTa electrode indicates that the oxidation of INO was faster at p-AMTa electrode than at bare GC electrode.

We also investigated whether the oxidation of INO at p-AMTa electrode is due to a diffusion-controlled or surface-confined process by recording the DPVs at different scan rates. The oxidation current of INO was increased while increasing the scan rates from 100 to 1000 mV s^{-1} (see Fig. S1 in Supplementary material). A good linearity was obtained while plotting the current against square root of scan rate with a correlation coefficient of 0.9988 (Fig. S1, inset). This indicated that the oxidation of INO was controlled by the diffusion process at p-AMTa electrode.