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A new reduction route of hypoxanthine and its nonenzymatic detection based on silver nanoparticles

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Abstract

The interaction between hypoxanthine (HX) and silver nanoparticles (NPs) is studied with electrochemical technique, together with UV–vis spectroscopy (UV–vis) method. Experimental results reveal that the usually silent carbonyl will be activated by the interaction between HX and silver NPs, which leads to a new electrochemical reduction route for HX. Meanwhile, a nonenzymatic detection method for HX is proposed. The reductive peak current is linear to the concentration of HX in the range of 1.0×10^{-6} to 1.0×10^{-5} mg/mL ($r = 0.998$) and 1.0×10^{-5} to 1.0×10^{-4} mg/mL ($r = 0.999$), respectively. The detection limit is 1.0×10^{-6} mg/mL. It is much more sensitive than the previous reported methods, which proves to be a potential way for HX measurements.

Keywords: Silver nanoparticle; Hypoxanthine; Catalysis; Electrochemistry; Detection

1. Introduction

Hypoxanthine (HX), which can be transformed to xanthine (Xa), then to uric acid (UA), together with O_2 to H_2O_2 through the enzyme xanthine oxidase (XOD) in vivo, is an important intermediate in the catabolism of purine nucleotides [1]. Its concentration directly reflects whether the catabolism is on track or not, thus it always acts as a mark molecule in the pathology of some process in human body [2]. And the determination of HX is of considerable importance in bioscience, clinic medicine as well as food industry, since the concentration of HX will increase when the body of animal decays [3]. To date, most of the reported researches to detect HX are based on XOD enzyme with the measurement of O_2 consumption or H_2O_2 formation [4,5], which has its inherent advantages such as high selectivity and sensitivity, and inevitable disadvantages of too much cost and inconvenient preparation for some well-known technique headaches [6].

Recently, with more knowledge of interface and molecular interaction as well as more skills in making chemically modified electrodes (CMEs), the application of nonenzymatic method to detect HX without the aid of XOD becomes possible and attractive. Zen's group reports a HX sensor based on nafion/lead-ruthenium oxide pyrochlore modified electrode and its successful application in evaluating fish freshness [7]. They also have reported another HX detector based on a preanodized nontronite-coated screen-printed electrode [8]. Wang et al. report a boron-doped diamond electrode to detect HX along with several other purines [9]. Toth and Cavalheiro investigate the effect of surface activation on the amperometric determination of HX and Xa by different mechanical/electrochemical methods [10]. Due to advantages of simplicity, low cost and stability without enzymes, as well as selectivity and sensitivity because of the special molecular interaction, nonenzymatic method is a good complementarity to the traditional enzymatic method and is expected to be a promising way.

Nowadays, nano-materials have been employed in the study of electroactive compounds due to their unique

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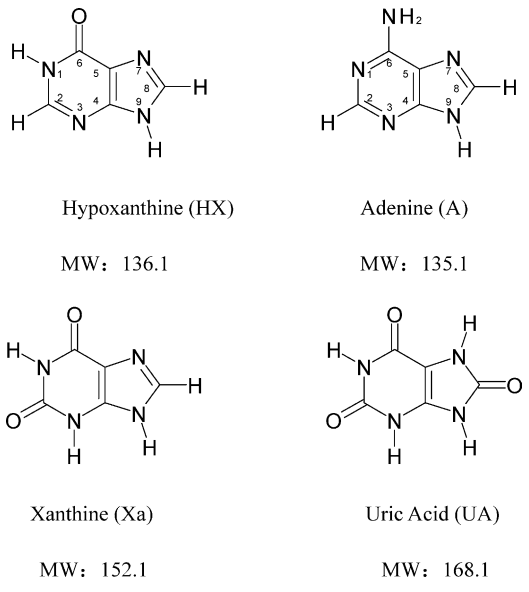
properties [11]. Here, we are particularly interested in silver NPs not only because silver NPs have high conductivity and well-demonstrated synthesis method [12], but also due to the fact that purine and its derivatives can adsorb at the surface of silver particles through specific sites and orientation which have been certified by surface-enhanced Raman spectroscopy (SERS) [13–16]. In this report, we present a new electrochemical reduction route of HX based on the interaction between silver NPs and HX. Consequently a nonenzymatic detection method for HX is proposed.

2. Experimental

Silver NPs (about 11 nm) were prepared according to the literature [12], and stored at 4 °C. HX, adenine (A), Xa and UA were from sigma (structures shown in Scheme 1). TiO_2 NPs (35 nm) and gold NPs (10 nm) were purchased from Nanjing Haitai Nano Company. Other chemicals were all of analytical grade. All solutions were prepared by double distilled water, which was purified with a Milli-Q purification system (Branstead, USA) to a specific resistance of >16 MΩ/cm.

The 20 μL silver NPs were spread on the previously mechanically cleaned and polished surface of pyrolytic graphite (PG) electrode, covered with an eppendorf tube overnight at room temperature, the PG electrode surface was then slowly dried and an even film formed. The modified electrode was thoroughly rinsed with double distilled water and was ready for use. When not in use, it was stored in a 0.1 M phosphate buffer solution (PBS) (pH 6.0) at 4 °C. TiO_2 NPs modified PG electrode was made in the same way as the silver NPs modified PG electrode.



Scheme 1. Structures of the purines.

The scanning electron micrograph was obtained with a SIRION scanning electron microscopy (SEM) (FEI, Holland). Cyclic voltammetry was performed with a VMP potentiostat (Perkin-Elmer, USA), in a conventional three-electrode cell in nitrogen atmosphere at 20 ± 0.5 °C. A saturated calomel electrode (SCE) and a platinum wire were employed as the reference and the counter electrodes, respectively. The working electrode was either a bare PG electrode or a modified PG electrode. UV–vis spectroscopy was performed with UV-2201 spectrometry (Shimadzu, Japan).

3. Results and discussions

Fig. 1 shows the morphology of the surface of the silver NPs modified PG electrode. The scattered little white dots are silver NPs, so the NPs which have not aggregated with an average diameter of 70 nm.

Cyclic voltammograms of silver NPs modified PG electrode in pH 6.0 PBS in the absence and presence of HX are depicted in Fig. 2. When HX is added to the solution, an irreversible reduction peak appears at the potential of −0.60 V, whereas no response can be observed in the absence of HX. The peak current increases with the concentration of HX which indicates that the peak at −0.60 V is owing to the reduction of HX. It has been known that the potential requirement for reduction of HX at PG electrode is −1.61 V [17], thus HX can be catalytically reduced with the help of silver NPs here, and there should be some specific interaction between silver NPs and HX.

Usually, the electroactive group of HX is considered to be the bond of C2=N3, which shows a reduction potential at −1.61 V [17]. In order to check whether the reduction peak of HX at −0.60 V is due to the C2=N3 or not, comparative experiments have been performed with A, Xa and UA. C2=N3 exists both in HX and A, so similar electrochemical responses should be obtained with these two species. Meanwhile, Xa and UA which have no C2=N3 group should

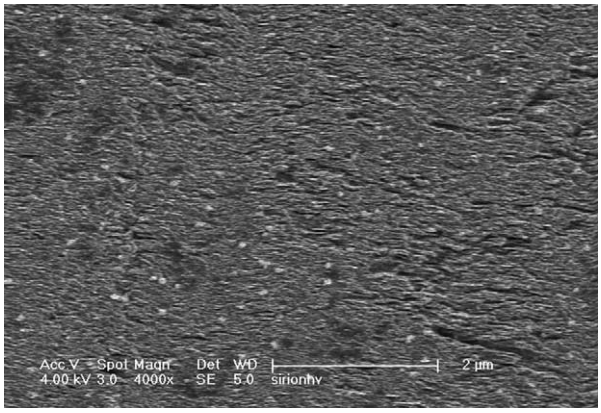


Fig. 1. The scanning electron micrograph of the surface of silver NPs modified PG electrode.

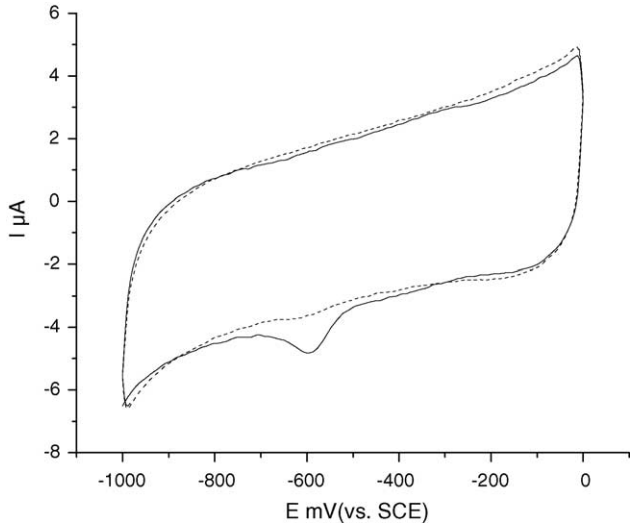


Fig. 2. Cyclic voltammograms obtained at a silver NPs modified PG electrode for 0.1 M PBS with pH 6.0 (dashed line) and the buffer containing 5×10^{-3} mg/mL HX (solid line); scan rate: 200 mV/s.

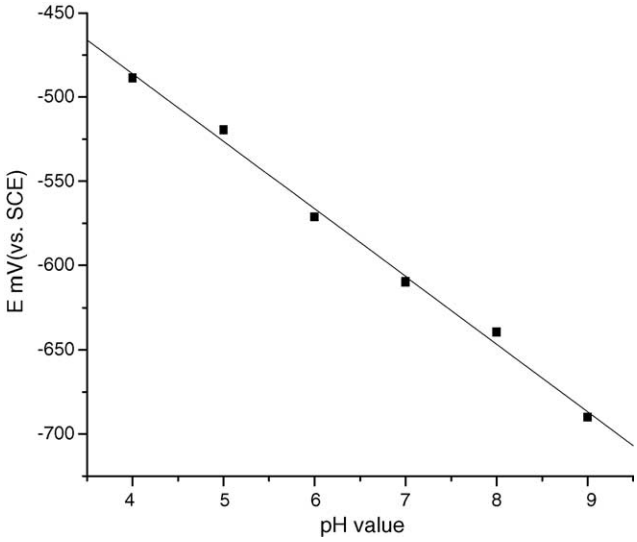


Fig. 3. Relationship between the reductive peak potential and pH value. Other conditions same as in Fig. 2.

be silent at the electrode. However, opposite results are observed. Similar electrochemical responses to HX appear with Xa and UA, whereas, there is no response with A. So, the usually regarded electroactive C2=N3 group becomes silent at silver NPs modified PG electrode. On the other hand, we should notice that the only difference between HX and A in their molecular structures is the group of C6 in their purine ring, and the carbonyl at C6 in HX is replaced by the amino at the same site in A. Since A does not exhibit electrochemical response at the silver NPs modified PG electrode, the new electroactive group of HX must be the carbonyl group at C6, which will get two electrons and two protons to be reduced to the hydroxyl.

Effect of pH upon the electrochemical behavior of HX has been investigated. The reduction peak of HX can be observed in all the pH range tested (4.0–9.0) and the peak potential shift negatively as the pH value increases. Fig. 3 shows the linear relationship, with regression equation: $y = -0.33\ -0.040x$, $r = 0.997$. Here, the slope 40 mV/pH is approximate to the theoretical Nernstian slope [18]. So the reductive process requires two electrons and two protons involved, which has also proved the above proposed.

There might be some specific interaction between HX and silver NPs. We have employed some other nano-materials, such as nano TiO_2 and nano gold, to prepare the modified electrodes so as to perform comparative experiments. Bare electrodes made of bulk Ag, Pt, or Au have also been used. Comparative studies show that no electrochemical response can be obtained for all these materials. Thus, the new reduction route of HX appears depending on its interaction with silver NPs.

UV–vis spectroscopic studies reveal that the molecules structure of HX has not been changed after the interaction with silver NPs. HX has two adsorption bands at 249 and 200 nm which represent π–π^* electronic transitions of the conjugate system in its purine ring [19,20]. A shift or disappearance of these two adsorption peaks indicates some structural changes of HX. However, as shown in Fig. 4, the UV spectra of HX mixed with silver NPs are totally the same as that of HX alone.

This new reduction route of HX may be utilized to detect its concentration directly without the help of XOD. Experimental results show that the reductive peak current of HX increases with the increase of HX concentration. As shown in Fig. 5, linear dependence between the peak current and the concentration of HX can be observed in the range of 1.0×10^{-6} to 1.0×10^{-4} mg/mL. The linear regression equations are $y = 0.43\ +0.039x$, $r = 0.998$ (1.0×10^{-6} to 1.0×10^{-5} mg/mL) and $y = 0.78\ +0.034x$,

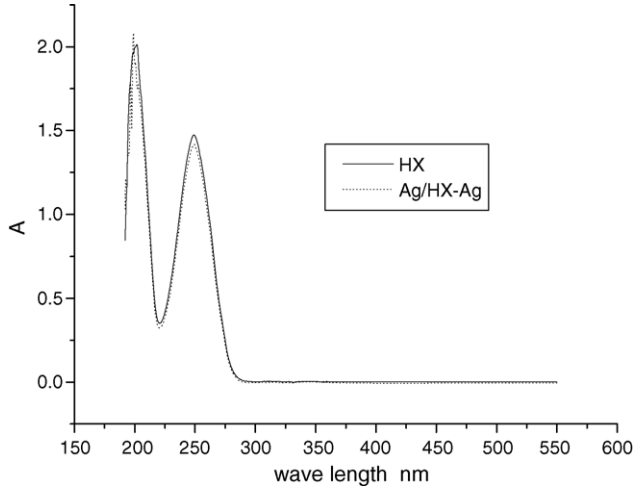


Fig. 4. UV–vis spectra of HX (solid line) and mixture of HX and silver NPs with $C_{HX}/C_{silver\ NPs}$ of 3.67/1 (dash line); HX concentration: 0.01 mg/mL.

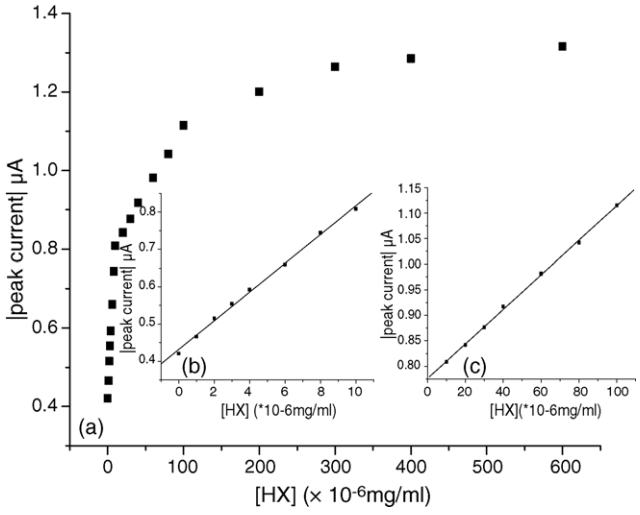


Fig. 5. Relationship between the reductive peak current of HX and its concentration (curve A): insets (curve B and C) are the calibration plots.

$r = 0.999$ (1.0×10^{-5} to 1.0×10^{-4} mg/mL), respectively. The detection limit is 1.0×10^{-6} mg/mL, which is lower than the previous studies [8,21,22]. Therefore, silver NPs modified electrode might be potentially applied as a new nonenzymatic detector for HX.

4. Conclusion

A new electrochemical reduction route is found for HX with silver NPs as an electrocatalyst. The interaction between HX and silver NPs ends the electrochemical silent life of carbonyl at C6 and produces a new reduction pathway of HX. And it can be applied to be a nonenzymatic detector for HX based on the Silver NPs tailored PG electrode with low cost and high sensitivity.

Acknowledgments

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