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A Highly Sensitive Amperometric Adenosine Triphosphate Sensor Based on Molecularly Imprinted Overoxidized Polypyrrole

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Abstract

Chemical Sensors and detectors play important roles in FIA systems. We have developed a molecularly imprinted polymer based amperometric sensor, which allows the detection of trace concentrations of adenosine triphosphate (ATP), aiming at food safety applications. The sensor has a small carbon electrode covered with an overoxidized polypyrrole film imprinted with an ATP molecule. The sensor operated in the triple pulse amperometric mode and was characterized in both the batchwise and flow-injection measurements. We have detected trace levels of ATP down to 5 nM without employing any separate preconcentration techniques.

Keywords Molecularly imprinted polymer, overoxidized polypyrrole, ATP, flow injection, sensor

1. Introduction

Contamination of food by microorganisms during preparation and processing is prone to lead to its poisoning and of great concern to the food industry [1]. To minimize the safety risk in the industry, the biological contamination has been detected by forming a colony of the target organism on a growth medium . However, the procedure is labor intensive and requires a period of days until the colony is fully developed and is ready for detection. Accordingly, an easy-to-use real-time sensing system is highly desired to detect such microbial contamination for on-site food-safety process control. To solve this issue, adenosine triphosphate (ATP) sensors have been proposed and already been available on the market. ATP is a molecule found in every biological system to work as an energy carrier [2] . Its presence in a target object either indicates that microbial contamination has already occurred or that there already exist favorable conditions for microorganisms to result in such contamination.

The commercially available ATP sensors, which rely on chemiluminescence resulting from the enzymatic reaction of luciferase with ATP [3], have several demanding conditions for on-site use. Those mainly arise from the instability of the expensive enzyme, which should be kept inactivated by freezing until used. Accordingly, it is highly desired to develop an easy-to-use and cost-effective real-time sensing system based on a working mechanism different from enzyme. Here, we report on an molecularly-imprinted-polymer (MIP) based sensor, which allows the detection of trace concentrations of ATP, aiming at food safety applications. The sensor was equipped with a small carbon electrode covered with an overoxidized polypyrrole film imprinted with ATP and operated in the triple pulse amperometric mode. We have examined the sensitivity and selectivity of the sensor in both the batchwise and flow injection experiments to find the detection limit of 5 nM without any separate preconcentration procedures.

2. Experimental

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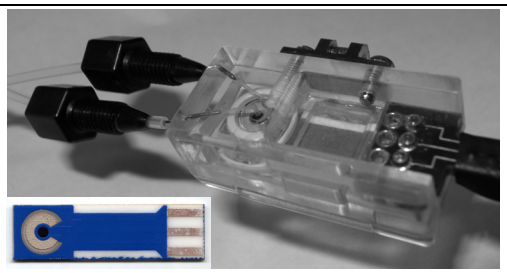

Fig. 1 The flow cell and sensor chip (left-bottom corner) used in this study.

2.1 Reagents

All the chemicals used here were of the reagent grade. Millipore grade water was used throughout.

2.2 Apparatus and Procedures

All the voltammetric experiments were performed with an Electrochemical Analyzer ALS Model 842B at ambient temperature. Flow injection experiments were preformed with a Model BF-30AS autosampler (Oji Scientific Instruments, Osaka). A sensor chip, Type AC1.W4.R1, was purchased from BVT Technologies (CZ) and used in both the batchwise and flow-injection studies (*See* Fig. 1). The sensor chip coaxially arranges a printed carbon working electrode (nominal diameter, 1.00 ±0.01 mm), and Ag/AgCl reference electrode and Pt/Au (15/85 wt%) counter electrode pads on a single ceramics base (7 mm × 25 mm). The chip was mounted in a wall-jet flow cell, Type FC2 (BVT technologies), for flow injection experiments. In the amperometric detection of ATP, triple pulse voltammetry was employed. In flow injection studies a flow rate of 2. 0 mL min^{-1} and an injection volume of 100 µL were used. The results obtained with our sensor were compared with those for a Lumitester PD-10N optical handheld sensor system (Kikkoman).

Polypyrrole doped with ATP was deposited on the carbon electrode by applying +0.98 V for 5 min from an aqueous solution containing 0.1 M pyrrole and 10 mM ATP. During polypyrrole film preparation and overoxidation treatment, all the potentials were referred to a separate Ag/AgCl/sat. KCl//

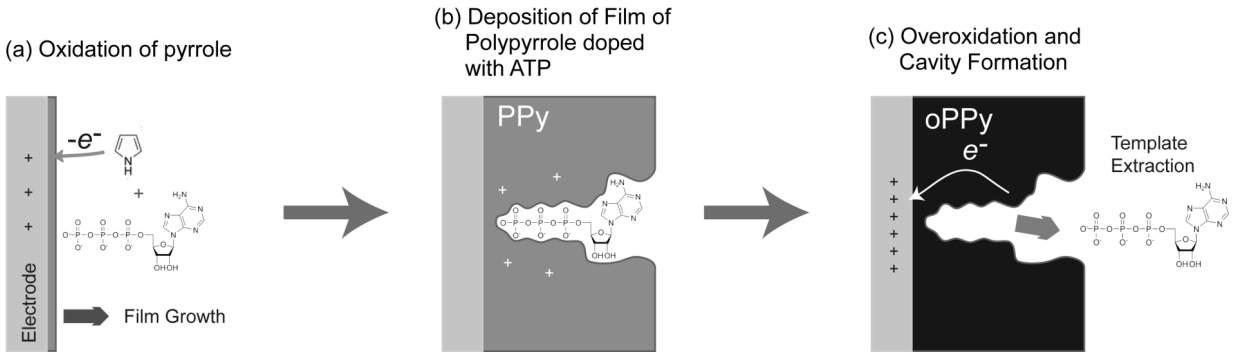

Fig. 2 Preparation scheme of oPPy(ATP): (a) Oxidative polymerization of pyrrole with ATP as a dopant on an electrode, (b) Prepared polypyrrole film doped with ATP, and (c) overoxidation of the polypyrrole film and the creation of the cavity complementary by dedoping ATP.

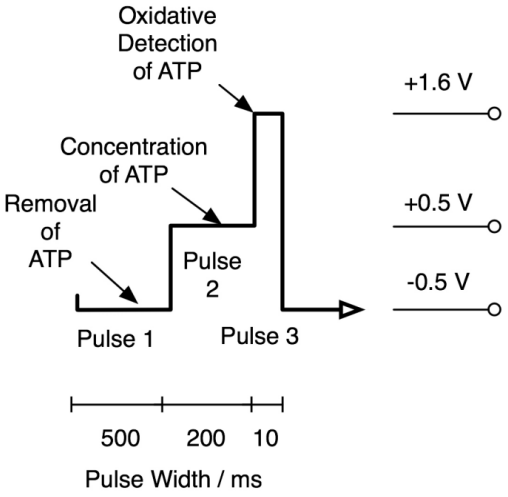

Fig. 3 Triple-pulse voltammetric wave form for ATP detection. The latter halves of the pulses 2 and 3 were sampled for the detection of the differential currents. The pulse sequence was repeated during the amperometric detection. The potential was referred to the Ag/AgCl pad on the chip shown in Fig. 1.

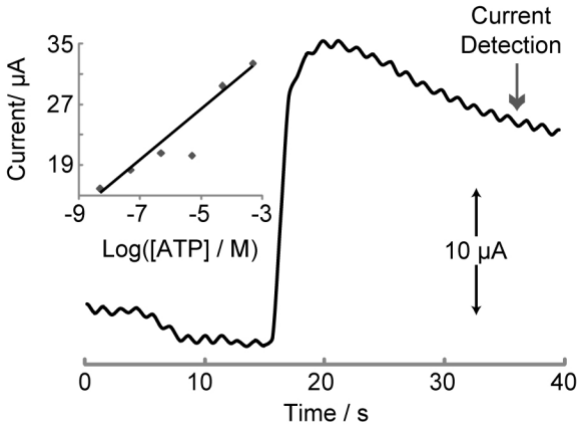

Fig. 4 Amperometric detection of 10^{-7} M ATP dissolved in a 90-mM phosphate buffer (pH 7.0). Inset: calibration curve.

electrode. The deposited film was then overoxidized in aqueous 0.1 M NaOH by cycling the potential twice from 0.0 V to +1.0V at a scan rate of 5.0 mV s^{-1}.

3. Results and Discussion

3.1 Formation of a MIP film with an ATP recognition site

Molecular imprinting is a technique utilizing tailor-made networked polymers for the recognition of specific analyte molecules, and the polymers can be classified as artificial enzyme systems [4,5]. Elsewhere we have presented the detailed recognition properties of overoxidized pyrrole (oPPy) MIP films as a new class of the MIP materials, which can effectively recognize several amino acids, aromatic sulfonates and bile acids [6-13]. In this study a polypyrrole (PPy) film was grown electrochemically on a carbon electrode from a pyrrole solution containing dopant, ATP (Fig. 2 a). The dopant, which is automatically included into the polymer matrix, served as a template of the recognition site. The resulting film (b) was oxidized again (overoxidized) in a basic media to exclude the dopant, which occurred due to removal of the positive charge in the polypyrrole matrix (c). The cavity left upon dedoping is therefore expected to have shape complementarity to the dopant (ATP). Curing of the film concomitantly occurring on dedoping is yet another useful property of this material for the formation of a robust recognition site [12]. The site created by overoxidation usually has high selectivity towards recognition of a template molecule (dopant). We have reported on the enantioselectivity of the oPPy films imprinted with several L- or D-amino acids to find that in most cases the selectivities were >10 [6-11]. Another benefit of this synthetic route is that all the preparation procedures can be sequentially performed on the electrode to create a molecular recognition site [10].

3.2 Voltammetric detection of ATP

Sensitive detection with thin film-based amperometric sensors requires effective removal of targeted analytes from the films before the next detection occurs. To achieve this we have adopted triple pulse amperometry (Fig 3). In this detection scheme, the electrode was first poised at –0.5 V. ATP acts as a four-valent anion in an aqueous medium and can be removed from the film by applying the negative potential. During the second pulse, ATP was effectively accumulated in the positively charged film by electrostatic interaction as well as by the affinity to the complementary cavity matching up with ATP. Finally, the third pulse oxidized the ATP molecule to detect its concentration. The currents resulting from the pulses 2 and 3 were differentially monitored for sensitive detection of the analyte. It has been suggested that the electrochemical oxidation of adenosine phosphates consists of relatively complex processes that include coupling reactions [14,15].

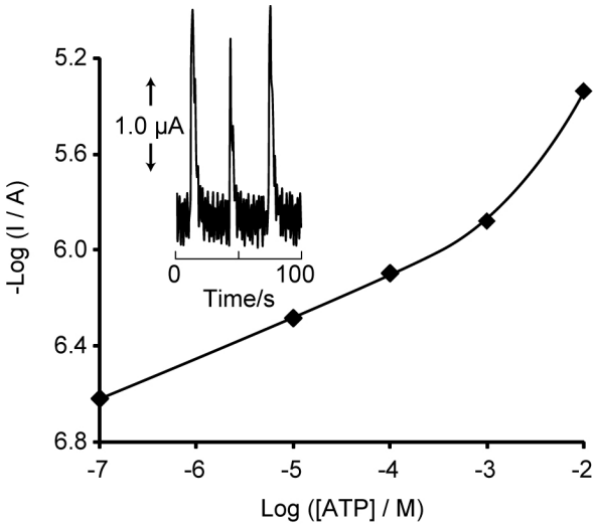

Fig. 5 Flow injection responses for ATP. Inset: response diagram for 100 nM ATP.

After the detection the potential was restored to -0.5 V to initialize the film, and the above pulsing sequence was repeated. Thus, the current was sampled every 0.71 s during a measurement and the whole response for a batchwise experiment is shown in Fig. 4. We have found an immediate increase in the current on the addition of ATP and recorded the differential current 20 s after the addition to construct the calibration curve given in the inset.

The curve demonstrates that the sensor has remarkably high sensitivity down to the nanomolar level without any separate preconcentration procedures, which are required by most electrochemical techniques for detection of trace species. The high sensitivity can be attributed to the affinity of ATP to the shape complementary cavity at the film surface and to the highly charged anionic structure of ATP, which can efficiently be concentrated into the film by the built-in pulsing sequence triple pulse amperometry provides. The detection limit of 0.1 mM at a bare carbon electrode indicates the achievement of a 10^4-fold increase in the sensitivity by the present technique. The sensor response showed a good correlation with those obtained with the luciferase based sensor.

The sensor responses for flow injection experiments are shown in Fig. 5. The flow experiments found high base current for an unclear reason when electrolyte was added to the carrier solution. To decrease the current, water without electrolyte was tentatively selected as the carrier. As a result, both the nonlinearity with respect to the ATP concentration and relatively poor S/N ratio were observed, arising from the low conductivity of the carrier at small ATP concentrations. Some optimization on the base electrolyte concentration would be required for practical application of this sensor in flow injection analyses.

We confirmed excellent selectivity of the oPPy film in the flow injection studies. The ratio of ATP to AMP in the peak height was as high as 16 at the oPPy(ATP) electrode, while it was only 1.1 at a bare electrode (AMP, adenosine monophosphate).

4. Conclusion

We have successfully developed an overoxidized polypyrrole based MIP sensor to detect trace level ATP aiming at on-site food-safety control applications, and some basic characteristics of this sensor performance have been discussed. The high sensitivity arose from the affinity of the cavity complementary to ATP and from the effective use of triple pulse amperometry, where the built-in initialization and accumulation modes were efficiently applied to the highly charged ATP molecule by controlling the electrostatic interaction. Some attempts on flow injection application have also been demonstrated.

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