Polymer Bulletin 46, 487–490 (2001)

Polymer Bulletin

Polycarbazole modified electrode; nitric oxide sensor

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Received: 26 February 2001/Revised version: 25 June 2001/Accepted: 25 June 2001

Summary

A sensitive and quick measurement of Nitric Oxide (NO) in real time is described using differential pulse voltammetry in aqueous solution. NO is estimated over electrochemically deposited polycarbazole conducting polymer modified platinum minielectrode of 0.0078 cm^2 geometrical area in deoxygenated 0.1 M phosphate buffer (pH 7.4) at 25°C. NO oxidation peak is observed during anodic scan at 0.65 V vs. Ag/AgCl. A linear dependency between NO concentration and anodic peak current is obtained in the standard addition of NO from 10 nM to mM range. The interference with ascorbic acid and dopamine are found to be negligible over polycarbazole modified electrode.

Key Words: Nitric Oxide, Conducting polymer, Polycarbazole, Differential pulse voltammetry.

Introduction

Nitric Oxide, an endogenously synthesized free radical is of great physiological and pathophysiological importance. It acts as a physiological messenger as well as a toxic compound. NO regulates vascular tone, acts as a neuronal signal in the gastrointestinal tract and central nervous system. Vascular endothelial cells have been found to produce a relaxant mediator, which was identified in 1987 as Nitric Oxide (1). From that time, free radical NO has emerged as an important signal molecule in mammalian physiology, including neurotranmission, vasodilatation and inflammation. NO estimation is important for the human health as it is involved in various biological processes, i.e., brain Ischemia, neurotransmission and immune regulation (2, 3).

Several methods e.g. chemiluminescence, UV-visible spectroscopic and electrochemical methods are reported for the detection of NO (4-6). However, there is still exists an emergent need to develop a cost effective, quick and sensitive sensing technique to detect the NO in real time because of its high reactivity and small half life (few seconds).

Electrochemical methods are most advantageous because of their speed and sensitivity. This is important because NO is highly reactive and is present at low concentrations (nM) in most of the biological systems. In addition, micro electrochemical sensors can be fabricated for measurement even directly into the biological systems. Several types of electrochemical sensors, e.g. conductometric and voltammetric sensors were reported for the detection of the NO in aqueous medium (6, 7). However, these were too complicated and selectivity were also not reported against major interfering ions like ascorbates and dopamine. The oxidation potential of ascorbates

and dopamine is close to the oxidation potential of NO and it also present in much higher concentration in comparison to the NO in biological fluid. Hence it is important to study the selectivity of the electrochemical sensor towards the NO in presence of ascorbates and dopamine.

In the present work we reported a sensitive, reliable and cost effective electrochemical sensing technique for NO. The major interference of ascorbic acid and dopamine in the biological samples were also overcome with the use of polycarbazole modified sensing electrode.

Experimental

Materials and Electrodes

NO gas (98.5 %) and Carbazole (98 %) were obtained from Aldrich Co., USA. Tetrabutylammonium perchlorate was obtained from Sigma Co, USA. G.R. grade hydrochloric acid (HCl), nitric acid (HNO_3) and dichloromethane were obtained from Merck, India. All other chemicals were used of A.R. grade and triple distilled, deionized and deoxygenated water was used for making the solutions.

Platinum minielectrode and platinum rod electrode were constructed in laboratory. Platinum minielectrode was constructed by embedding the fine platinum wire in the glass rod, grinned and polished to expose the platinum micro tip of 7.8×10^{-3} cm^2 geometrical area. Platinum rod electrode was constructed by embedding the platinum wire in the glass rod with the exposed length of 1 cm. Ag/AgCl reference electrode was purchased from Orion, USA. All the electrochemical work was done over computer interfaced E & G PAR 273 A Potentiostat Galvanostat, Princeton, USA.

Electrochemical Synthesis of Polycarbazole

Electrochemical synthesis of polycarbazole over Pt micro-electrode was carried out under potentiostatic and potentiodynamic conditions as described earlier (8-9). Polycarbazole was formed in non-aqueous electrolyte of dichloromethane having 0.1M tetrabutylammonium perchlorate and 60 mM carbazole. Potentiodynamically polycarbazole was formed by sweeping the potential in the range of –0.2 V to 1.4 V vs. Ag/AgCl at the scan rate of 50 mV/s and under potentiostatic condition, 1.4 V vs. Ag/AgCl was applied to get the polycarbazole film over the platinum micro-electrode.

Standard solution of NO

NO standard solution of typical concentration of 1.8 mM (10) was prepared in the laboratory by passing NO gas through deoxygenated 0.1M phosphate buffer (pH 7.4) solution at 25°C for 30 minutes. The PB was deoxygenated by purging argon gas for 30 minutes prior to dissolution of NO.

Differential Pulse Vollammetry

NO was estimated in deoxygenated 0.1M phosphate buffer (pH 7.4) by differential pulse voltammetry over a bare platinum minielectrode as well as polycarbazole modified electrode. The positive potential scan was done in the range of 0.0 V to 1.0 V vs. Ag/AgCl by imposing the differential pulse mode with scan rate of 20mV/s. An anodic peak corresponding to NO oxidation was observed at 0.65 V vs. Ag/AgCl. The varying concentration of NO was added and the experiment was repeated after each addition. Peak current was recorded with the variation of NO concentrations. The calibration curve was plotted for peak current vs. log (NO concentration).

The interference was studied in presence of ascorbic acid and dopamine (higher to body level) for a typical concentration of 0.1 M NO by scanning DPV in the same potential range.

Results and Discussion

Nitric oxide oxidation was studied in phosphate buffer solution over a bare platinum minielectrode along with the platinum auxiliary electrode and a double junction Ag/AgCl reference electrode. A broad oxidation peak appeared at 0.65 V vs. Ag/AgCl for 0.1 mM NO concentration, as shown in Fig. 1 (b). The measurements were done for the lower concentrations of NO by using standard addition method. NO oxidation peak did not appeared below 10^{-6}M, moreover the peak current vs. NO concentration did not give the linear plot even in mM to µM range. The platinum minielectrode was modified with the polycarbazole conducting polymer and was used as a sensing electrode in the same configuration as bare platinum minielectrode was used. The background current was recorded as shown in Fig. 1(a). NO sample of 0.1 mM and 10 nM concentration was prepare and a sharp anodic peak, with relatively higher current was observed at the 0.65 V in the positive potential scan as shown in Fig. 1 (c) and inset of the Fig. 1 respectively. The polycarbazole matrix provided the larger electrode area and hence an enhancement in the NO oxidation peak current was observed.

An unpaired electron is present in the outer π^* molecular orbital of NO, which is having a low ionization potential. The NO molecule can be easily oxidised as nitrosonium ion (NO^+) on applying an anodic potential above 0.6 V vs. Ag/AgCl in aqueous medium.

In the electrochemical detection of NO in the biological samples the major interference is caused by the dopamine and ascorbic acid. The interference for both the chemicals were eliminated by the use of the polycarbazole modified electrode. The ascorbic acid oxidation was suppressed at the polycarbazole matrix and dopamine peak appeared at the 0.25 V vs. Ag/AgCl quite far from NO peak (11). The polycarbazole electrode is selective for cations or neutral molecules probably because of doping of anions into the matrix, and hence only neutral molecules are cations permeate in to the matrix (12). At the pH of the medium employed (7.4 pH, buffer), ascorbic acid exists in the anion form (pka=4.17) and consequently repelled by the polycarbazole film. NO was estimated over the modified electrode in the range of 10 nM to 0.1 mM. A linear dependency between the NO concentration and the anodic peak current was obtained, with the detection limit of 50 nM as shown in the Fig. 2.

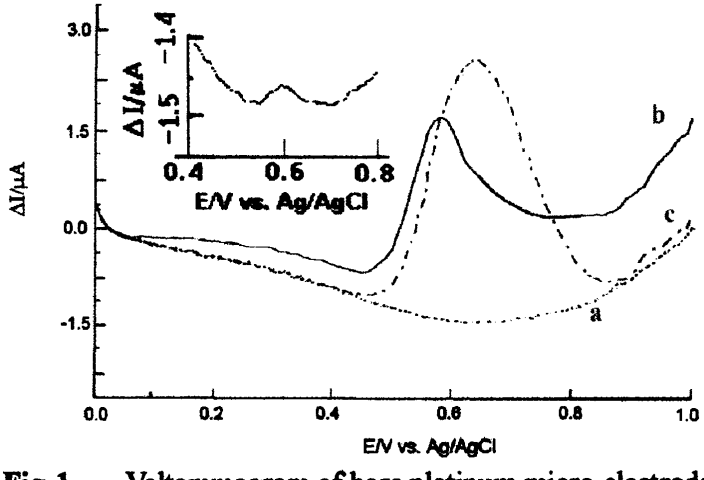

Fig.1. Voltammogram of bare platinum micro-electrode in 0.1 mM NO in 0.1M phosphate buffer of pH 7.4 (b), polycarbazole modified platinum micro-electrode in 0.1M phosphate buffer (a) and in 0.1 mM NO in 0.1M phosphate buffer of pH 7.4 (c). Inset showes the voltammogram of 10 nm NO in the same buffer at Polycarbazole modified electrode.

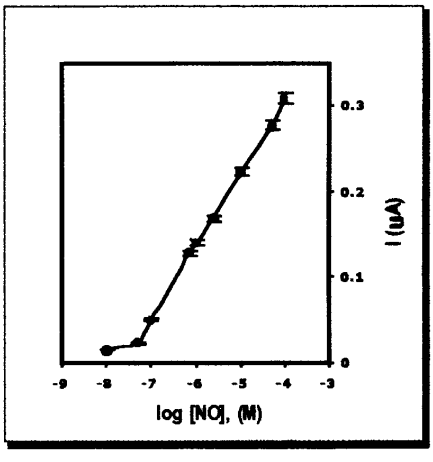

Fig. 2. Calibration plot for NO oxidation peak current vs. log of NO concentration.

Conclusion

Nitric oxide was quantified in the aqueous solution up to 50nM concentration using polycarbazole modified electrode. The NO detection was described in the wide rage, 10nM to 0.1 mM concentration without any interference of ascorbic acid or dopamine. It appears from above preliminary results that a polycarbazole modified electrode can be used as a simple material for the design of sensitive and cost effective electrochemical sensor for NO in biological matrix.

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