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Ion Recognition and Analytical Application of a Fibroin Modified Electrode

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Abstract: A novel fibroin modified electrode with ion recognition was reported. The membrane with isoelectric point of pH 4.5, was modified on graphite and carbon fiber electrodes. The pH-responsive ion recognition of the modified electrode was investigated by use of some neurocompounds. The fibroin carbon fiber electrode has been used for in-vivo determination.

Keywords: Silk fibroin, pH-responsive ion selectivity, chemically modified electrode.

The silk fibroin protein membrane is an amphoteric ion–exchange membrane composed of both weak acidic and weak basic groups. The positive or negative charge of the membrane depends on the pH of solution. In this paper, the fibroin is coated on the surface of graphite and carbon fiber electrodes. Some neurocompounds, such as dopamine (DA), 3, 4-dihydroxyphenylacetic acid (DOPAC), 5-hydoxytrytamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA), are chosen to investigate pH-responsive ion selective recognition. A fibroin carbon fiber electrode was used for *in-vivo* determination of some neurotransmitters in rat brain.

Silk fibroin solution is prepared from Chinese cocoon. The silk thread is degummed, and subsequently dissolved in $CaCl_2/C_2H_5OH/H_2O$ solution (1/2/8 mol ratio). After dialysis the final concentration of fibroin solution is 10 g/L.

A graphite electrode (7.5 mm of diameter) is coated with 20 μL of silk fibroin solution (10 g/L), and then immersed in methanol solution for 20 min to make the membrane insoluble. A carbon fiber electrode (7 μm of diameter) activated by the electrochemical method[1], is dip-coated in fibroin solution (10 g/L), and pretreated in methanol solution. A BAS-100B/W analyzer is used for *in-vitro* and a BAS CV-37 analyzer is for *in-vivo* experiments.

The fibroin membrane is amphoteric, and its isoelectric point is at pH 4.5[2]. When the solution pH is lower than 4.5, the membrane reacts with H^+ and is charged positively. When the pH is higher than 4.5, the membrane reacts with OH^- and is charged negatively. So, the fibroin membrane modified electrode could be used to detect cations or anions

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selectively in different pH. In the experiment, two PBS solutions with pH 3 and 7.4 were selected, and two compounds of DA and 5-HIAA were chosen as probe molecules to examine pH responsive ion recognition of the modified electrode. Both DA and 5-HIAA showed voltammetric responses in a pH 3 or 7.4 solution at a bare graphite electrode. However, only a voltammetric peak of DA in a pH 7.4 solution, and a peak of 5-HIAA in a pH 3 solution were observed at a fibroin membrane electrode (**Figure 1**). It demonstrates that a negatively charged membrane electrode rejects anion of 5-HIAA, and attract positively charged DA to the electrode at pH 7.4. In the same way, a positively charged membrane electrode rejects DA and detects anion of 5-HIAA at pH 3. The ion recognition of silk fibroin membrane electrode depends on pH of solution.

In this experiment, 1×10^{-5} mol/L DA, 5-HT, DOPAC, 5-HIAA and 1×10^{-4} mol/L AA were mixed in a pH 7.4 PBS solution, in which a bare graphite or membrane modified electrode was immersed. Peak potentials of above compounds in differential pulse voltammetry are shown in **Table 1**. All of these compounds showed sharp voltammetric peaks in the bare electrode. However, some peaks are overloaded, such as the peak potentials of DA, DOPAC and AA are at about 0.18 V, and the peak potentials of 5-HT (0.35 V) and 5-HIAA (0.31 V) are very close. These compounds interfere with each other seriously at a bare electrode in the mixed solution with all five species, while there were only two well-defined peaks of DA and 5-HT were observed at the modified electrode (**Figure 2**). It means that anions of AA DOPAC and 5-HIAA are rejected in a pH 7.4 solution, only DA and 5-HT are detected by the negatively charged electrode. There is a linear relationship between peak current and concentration for these two compounds.

Figure 1 Voltammograms of DA and 5-HIAA at bare and fibroin electrode in different pH solutions

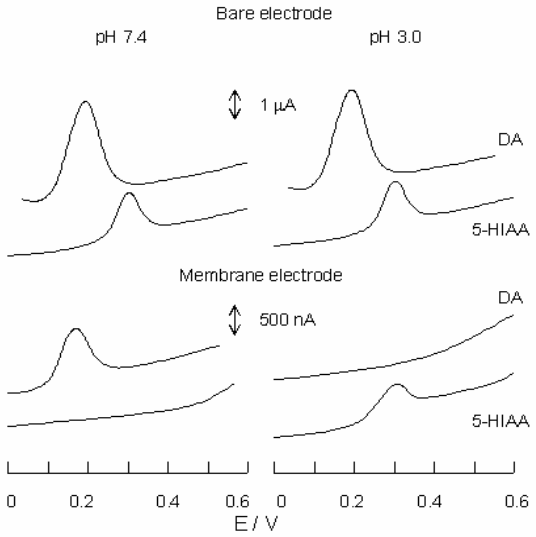
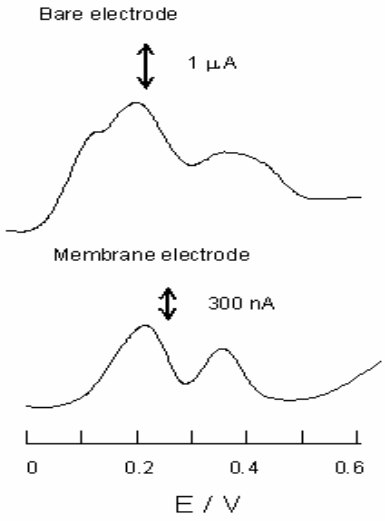


Table 1 Peak potentials of some compound in differential pulse voltammetry

Electrode	*Peak potential (V)*				
	DA	DOPAC	5-HT	5-HIAA	AA
Bare graphite	0.18	0.18	0.35	0.31	0.15
Fibroin membrane	0.18	—	0.35	—	—

Figure 2 Voltammograms at bare and fibroin electrode in a solution with DA, DOPAC, 5-HT and 5-HIAA



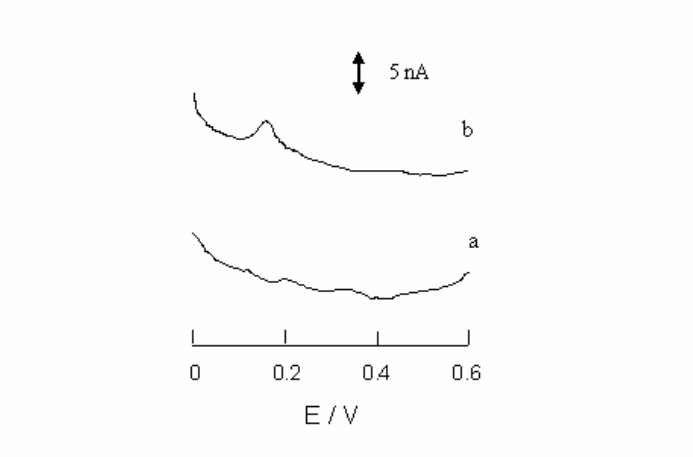
Normal electrodes are easy to be poisoned during electrochemical determinations, because of complicated species in sample solution. Protection effect of the membrane was estimated by adding 1×10^{-4} g / mL of albumin into a DA solution. The membrane modified electrode kept its sensitivity, while a bare electrode lost 38 % of peak current, suggesting that the fibroin membrane could prevent from adsorption of some protein on electrode surface.

In-vivo experiments were carried out, as reported in previous work[3]. A Sprague-dawley rat was anesthetized with 120 mg choral hydrate at first, the head was then fixed in a David Kopf stereotaxic instrument. After removing the surface skin, a hole (5 mm diameter) was made by a dental drill and cleaned by saline water, the residual blood and bone were removed carefully to prevent the electrode from being poisoned and damaged. A fibroin carbon fiber electrode was placed in burr hole, a stainless steel screw on the skull was used as a counter electrode, and all three electrodes were connected to the BAS CV-37 analyzer. Voltammetric responses were recorded every 5 min, after a stable base line was obtained.

There was no obvious peak observed at the fibroin electrode (**Figure 3 a**) while the peaks of AA, DOPAC, 5-HIAA, uric acid (UA) and homovanillic acid (HVA) arose at a bare electrode (not shown). It implies that anionic compounds of AA, DOPAC,

5-HIAA, UA and HVA in rat brain have been rejected out of the fibroin electrode, and the concentrations of cationic neurotransmitters DA and 5-HT are too low to be detected. An increasing peak of DA (0.18 V) was recorded (**Figure 3 b**) after an injection of DA delivering drug – amphetamine (2 mg/kg). It means that the positively charged compounds in body fluids could be detected at the fibroin modified electrode.

Figure 3 *In-vivo* voltammograms at the fibroin electrode in rat brain without injection (a), 20 min after an injection of amphetamine (b)



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