Surface Electrochemical Measurement of Acetylcholinesterase Activity: Application to Monitoring a Peptide Hormone with Enzyme Immunoassay System

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The determination of B-type natriuretic peptide (BNP) in blood is of diagnostic value in heart diseases. The blood BNP levels in normal conditions are extremely low, ca. 20 pg mL^{-1}, but they elevate to be > 2 ng mL^{-1} for patients diagnosed with severe congestive heart failure (CHF). To provide cost-effective treatment for patients with CHF, rapid and accurate differentiation of CHF from other cause of dyspnea must be accomplished: the determination of BNP provides useful information for the purpose.

We have previously developed a novel coulometric method for measuring the activity of acetylcholinesterase (AChE, EC 3.1.1.7) with higher sensitivity than conventional amperometric method by 100 times. Thiocholine, which was produced through the AChE-catalyzed reaction, chemisorbed on a silver electrode surface and desorbed cathodically through one-electron path. Thiocholine in an AChE-assaying solution could be accumulated on the electrode surface through the chemisorption. The accumulation resulted in the amplified coulometric response for the desorption and in the enhanced sensitivity, similarly to the case of adsorptive stripping electrochemical measurements. Such a method for measuring the enzyme activity would be useful for the enzyme immunoassay of antigen with low concentrations.

In this paper, the above AChE-determining method was applied to the detection of BNP, based on the enzyme immunoassay using an AChE-labeled anti-BNP antibody. The peptide in the concentration range from 20 pg mL^{-1} to 200 pg mL^{-1}, which fit in with the blood BNP levels, could be measured within an hour.

The AChE activity was determined based on the following procedure. The test solution used was 0.1 M phosphate buffer solution (PBS, pH 8.0) containing 5 mM acetylthiocholine. The dialysis membrane-covered silver electrode was immersed in the solution immediately after the addition of AChE to chemisorb thiocholine produced through the enzymatic reaction. During the chemisorption process, we applied a negative potential of -0.7 V (vs Ag/AgCl) unless otherwise stated. Then the dialysis membrane on the electrode surface was removed, and the electrode was rinsed with water. Finally, the electrode was transferred into 0.1 M KOH and the thiocholine that had been chemisorbed on the electrode surface was electrochemically desorbed to measure the charge for the desorption process in relation to the AChE activity.

A gold disk (diameter, 1.6 mm) was modified with cysteamine by soaking the disc in the modifier solution (0.1 mM) for 12 h at room temperature. The cysteamine-modified disk was soaked in 10 mM PBS (pH 8.0) containing 0.1 mg mL^{-1} 1-ethyl-3-(3-dimethylaminopyopyl)carbodiimide, and 2.5 mg mL^{-1} BNP was added to be attached covalently to the disk. After the incubation for 1 h, the BNP-attached disk was rinsed with the 10 mM PBS and stored at pH 8.0 at 4 ˚C. AChE-labeled anti-BNP antibody was synthesized as follows. The antibody modified with maleimide moieties was prepared by adding anti-BNP antibody (0.4 mg mL^{-1}) and Sulfo-SMCC (0.4 mg mL^{-1}) successively into 0.1 M PBS (pH 8.0), and incubating the solution for 1 h at room temperature. The resulting solution was ultra-filtered to remove excess Sulfo-SMCC. Similarly, AChE introduced -SH group was obtained by reacting the enzyme (1 mg mL^{-1}) with s-acetylmercaptosuccinic anhydride (0.3 mg mL^{-1}) in 0.1 M PBS (pH 8.0) for 10 min at room temperature. The solution was ultra-filtered to remove the unreacted modifier. Then the maleimide-modified antibody and thiol-modified AChE were mixed with the molar ratio of 1:0.7, and the mixture was incubated for 1 h at room temperature so as to synthesize AChE-labeled anti-BNP antibody.

BNP was determined through the following procedure. A certain amount of BNP was added to 0.1 M PBS (pH 8.0, 10 mL) containing 25 ng mL^{-1} AChE-labeled anti-BNP antibody to undergo the immunological reaction (incubation period, 30 min; at room temperature). Then the BNP-modified gold disk was immersed into the antigen/antibody solution for 30 min, so that unreacted AChE-labeled anti-BNP antibody was attached on the gold disc. Then the gold disc and the silver electrode pretreated was immersed into 0.1 M PBS containing 5 mM acetythiocholine (pH 8.0, 0.5 mL), then AChE activity on the gold disc was measured according to the procedure described in the previous section.

The increase in the BNP concentration in the sample to be measured caused the decrease of the unreacted antibody concentration, and that of the AChE activity on the BNP-attached gold disk as can be understood. Hence the electrode response for the reductive desorption of thiocholine is considered to decrease in association with the increase in the BNP concentration. The high sensitivity for the AChE activity shown in the previous section would result in the sensitive detection of the amount of the enzyme-labeled antibody on the gold disk and, therefore, the concentration of BNP. The charge for the desorption process was decrease with the increase of the analyte concentration, as expected. It should be emphasized here that the addition of BNP < 200 pg mL^{-1} brought about discernible decrease in the electrical charge: the lower detection limit was 20 pg mL^{-1}. This indicates that our method can be applied to measure blood BNP level for the diagnosis of heart diseases.