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THE ELECTRICAL METHOD OF INVESTIGATION OF THE ANTIGEN-ANTIBODY AND ENZYME-ENZYME INHIBITOR REACTIONS USING CHEMICALLY MODIFIED ELECTRODES

Naoto YAMAMOTO, Yoshikatsu NAGASAWA, Sadanobu SHUTO,
Masanobu SAWAI*, Tadamitsu SUDO*, and Hiroshi TSUBOMURA
Department of Chemistry, Faculty of Engineering Science,
Osaka University, Toyonaka, Osaka 560
* Research Laboratories, Teikoku Hormone Mfg. Co., Ltd.,
Kawasaki-shi 213

The shift of potential of electrodes chemically modified with biologically active substances (antigen or enzyme) due to their reactions with their counterparts (antibody or inhibitor) have been measured. It has been proved that such electrical measurements can serve as a new technique for the very sensitive detection of biologically active substances.

We have developed an entirely new electrical method to detect biological substances such as antigens, antibodies and enzymes by means of measuring the electric potential of some chemically-modified electrodes. The working electrodes are made of titanium wire chemically modified either with an antigen or an antibody by use of cyanogen bromide using a similar method of Weetall et al.[1] Human chorionic gonadotropin (hCG)[2] and anti-hCG immunoglobulin obtained from the immunized rabbits are used as antigen and antibody, respectively. The reference electrode was a titanium wire chemically modified with urea. These two electrodes are immersed in an 0.05 M veronal buffer solution, 6 ml, at pH of 8.6 and 35°C as shown in Fig. 1, and the potential between them is measured with a vibrating reed electrometer.†

It was observed that the electrode potential between the anti-hCG modified electrode and the reference electrode began to shift exponentially toward the positive when an hCG solution was added into the veronal buffer solution as shown in Fig. 2. No potential shift was observed for the anti-hCG electrode by adding any other proteins. The potential shifts can therefore be attributed to the specific reaction between hCG and anti-hCG at the modified electrode surface. On the other hand, the potential of the hCG modified electrode was observed to shift toward the negative by addition of an anti-hCG solution.

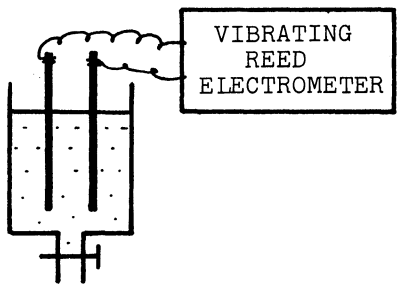

Fig. 1 Apparatus

Since the amount of antigen or antibody attached at the working electrode surface was very

small compared with that of the reactant added into the veronal buffer solution, the concentration of the reactant is considered to stay constant during the reactions. Assuming that the change of potential is proportional to the amount of the reaction product on the electrode surface, the electrode potential curve as shown in Fig. 2 can be explained well according to the first order reaction kinetics.

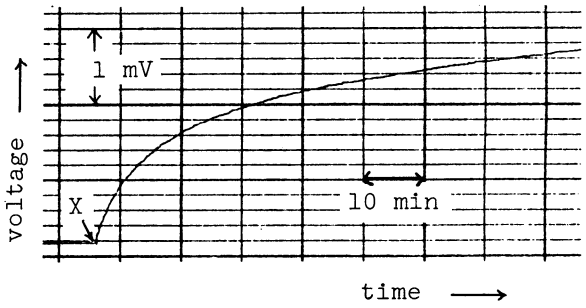

Fig. 2
The shift of the potential between the anti-hCG electrode and the reference electrode by addition of hCG at time as indicated by X. The final concentration of hCG was 3.3 μg/ml.

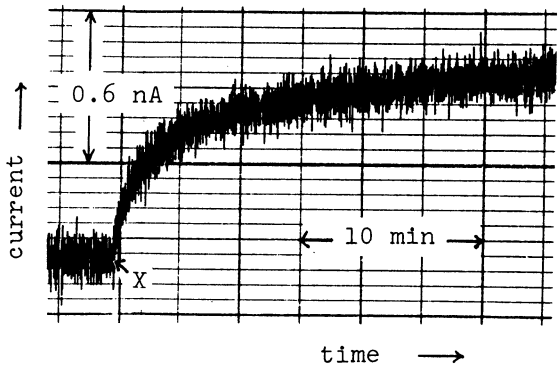

Fig. 3
The change of current between the anti-hCG electrode and the reference electrode by addition of hCG at X. The final concentration of hCG was 4.4 μg/ml.

Similar shifts of potential were also observed using a voltmeter having an input impedance of 10^6 Ω, but the signal became about 20 times as small as that measured by the vibrating reed electrometer. The output impedance of the electrodes was presumed to be of the order of 10^9 Ω. The reactants on the modified electrodes can be removed by immersing them into an 0.05 N hydrochloric solution cooled at 5°C for 2 min and the electrode can be used repeatedly. Similar electrode potential shifts have been observed for a complex formation reaction of trypsin with aprotinin, its enzyme inhibitor.[3)]

The current arising from the reaction between hCG in solution and an anti-hCG electrode has also been measured using either the vibrating reed electrometer or Keithley Model 414S picoammeter (Fig. 3). The current response is rather quick and noisy compared with the electrode potential shift. The analysis of the result obtained from the current measurement in connection with the electrode potential will be discussed in detail later.

The modified electrode techniques as described above will have a wide applicability as a highly sensitive sensor of biologically active substances. It will also offer interesting informations on the electrical feature of in vivo reactions.

Reference

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† The solution was stirred during the measurements.

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