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The Potentiometric Detection of Complex Formation between IgG and anti-IgG Using a Chemically Modified Tantalum Electrode

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Tantalum electrodes were chemically modified with anti-IgG to detect IgG (immunoglobulin G) or with IgG to detect anti-IgG by using cyanuric bromide. Changes in the potential as a result of the antigen-antibody reaction can be explained by a simple first-order kinetics equation, while the polarity of the changes at various pH's can be explained in terms of the charge transfer between anti-IgG and IgG in a way similar to that shown in previous reports for the case of Ti electrodes. The surface modification was confirmed by Auger Electron Spectroscopy (AES).

Many kinds of electrochemical methods have been investigated in order to detect biological substances.[1-7] Among them, the potentiometric detection method using chemically modified electrodes,[4-7] developed in our laboratory, is unique in that the responses depend directly on the complex formation on the electrode surface, and so quantitative detection can be achieved without using any enzymes which produce H_2O_2, NH_4^+, or other electrochemically active species. For example, antigen (hCG) was detected[4-6] by the use of a titanium electrode chemically modified with antibody (anti-hCG) without being marked by any reactive chemicals like peroxidase. In a similar way, other immunological substances, enzymes (e.g., trypsin), or enzyme inhibitor (e.g., aprotinin) were detected by the use of modified titanium or tungsten electrodes.[6,7]

The stability in the potential of these chemically modified electrodes critically influences the detection limits, reliability, accuracy, and precision of the potentiometric determination. In a recent attempt, we coated a tungsten electrode with a plasma polymerized film prior to the chemical modification in order to improve its electrochemical stability.[7] Tantalum/tantalum oxide electrodes were foumd by Lerner et al.[8] to be stable as implanted stimulating electrodes.

In this paper, a tantalum electrode chemically modified with an antibody (anti-human IgG-Fc, momoclonal) and an antigen (human IgG) were investigated to determine their possible use as a potentiometric sensors for an antigen (human IgG) and an antibody (anti-human IgG) respectively. The kinetic properties and the pH effects on the response of the tantalum electrodes were also investigated for the further elucidation of the potential change.

Electron spectroscopic analysis techniques such as X-ray photoelectron spectroscopy (XPS), Auger electron spectroscopy (AES), and electron energy-loss spectroscopy (ELS) are efficient for analyzing the surfaces of chemically modified electrodes. The surfaces of graphite, tin oxide, and indium oxide electrodes chemically modified with hydroxymethylferrocene (HMF) or 1,1′-bis(hydroxymethyl)ferrocene (BHMF) by using cyanuric chloride were investigated by means of XPS by Yacynych and Lin.[9,10] In this report, the chemical modification of a tantalum surface by using cyanuric bromide was monitored by AES.

Experimental

Material. Lyophilized IgG from human serum (98% pure) was obtained from the Green Cross Co. The anti-human IgG-Fc, monoclonal antibody, and lyophilized bovine serum albumin (BSA) were obtained from Wako Pure Chemical Industries Ltd. Tantalum sheets, 0.1 mm thick and 99.9% pure, for the surface investigations, and a tantalum rod 4 mm in diameter (99.95% pure) for use as an electrode were obtained from the Japan Lamp Industrial Co., Ltd.

Preparation of Tantalum Electrodes. Figure 1 shows the structure of a typical tantalum electrode for the potentiometric measurement; it consists of a tantalum tip 4 mm in diameter, a tantalum rod 2 mm in diameter and 40 mm in length, a stainless steel nut, and a chlorotrifluroethylene (CTFE) pipe. The tantalum tip was washed by ultrasonifi-

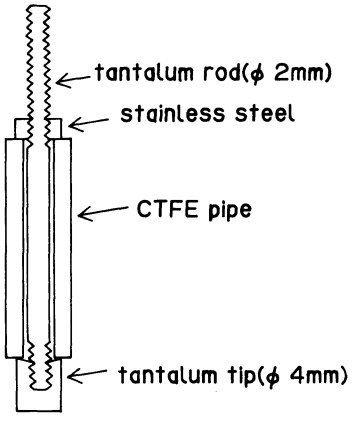

Fig. 1. The structure of a tantalum electrode.

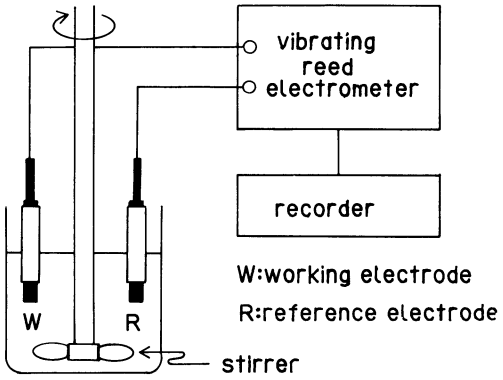

Fig. 2. A schematic illustration of the apparatus for the potentiometric measurement.

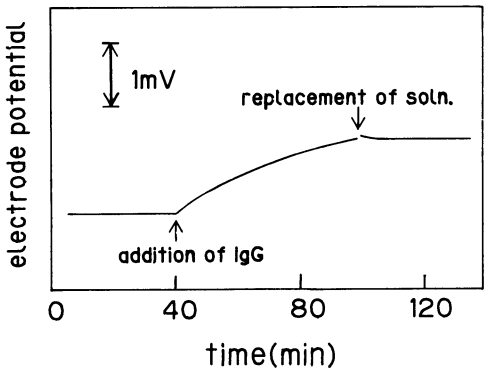

Fig. 3. The potential change of an anti-IgG-Fc electrode caused by addition of human IgG(2.5×10^{-8} M) in veronal buffer (pH 8.6).

cation in 2 M hydrochloric acid (1 M=1 mol dm^{-3}), a 2 M sodium hydroxide solution, acetone, and then in purified water, each for 10 min. Then the tip was treated with a ca. 500 V low-frequency plasma discharge in a vacuum chamber for 20 min under an oxygen atmosphere of 0.15 Torr (1 Torr≈133.322 Pa) before chemical modification.

Chemical Modification. The chemical modification of the tantalum electrode surface was performed by a method similar to that of March et al.[11] The tip was chemically activated in a stirred aqueous solution containing 1.2 M sodium carbonate, 0.5 M cyanogen bromide, and 1 M acetonitrile. Then, the tip was immersed in a borate buffer solution (pH 8.1) containing a 0.02 wt% anti-human IgG-Fc monoclonal antibody for 90 min to form an anti-IgG-Fc electrode. A human IgG electrode was made similarly in a buffer solution containing 0.02 wt% human IgG instead of anti-human IgG-Fc. A BSA electrode, used as the reference, was similarly made in a buffer containing 0.02 wt% BSA instead of human IgG. At the end of the chemical modification, all the electrodes were immersed in a borate buffer solution (pH 8.1) containing 0.7 M urea for 30 min in order to block the unreacted part of the electrode surface.

Potentiometric Measurement. The potential between the working and reference electrodes were measured by using a vibrating reed electrometer, TR8411, of the Takeda Riken Industry Co., Ltd. The experimental set-up is shown schematically in Fig. 2. The solution in the measuring vessel is a 15 ml veronal buffer solution (pH 8.6, 0.05 M) kept at 30°C and stirred constantly. The pH effect was measured in Britton Robinson buffers[12] whose ionic strength was adjusted to 0.1 by diluting with water,[13] although it is known from our previous work that the ionic strength of a solution scarcely affects the electrode response.

Surface Analysis. The structure of the tantalum surfaces at each step of the chemical modification were investigated by AES using a PhiModel 10-155 cyrindrical mirror analyzer (CMA) at an electron-beam voltage of 3.0 keV and with a current of 10^{-6} A. The differential spectra were obtained by a 6 V modulation. Tantalum sheets (13 mm×13 mm) were treated in the same manner as with the anti-human IgG-Fc electrodes and were then used as specimens for the surface analysis.

Results and Discussion

Potentiometric Measurement. Compared with the titanium electrode used in our previous work, the tantalum electrode is electrochemically more stable, and the time necessary for stabilizing the electrode potentioal for the tantalum electrode became about one tenth of that for the titanium electrodes.

As is shown in Fig. 3, the potential of an anti-human IgG-Fc electrode changes irreversibly to the positive upon the addition of human IgG to the veronal buffer solution at pH 8.6. The potential did not, however, change after the replacement of the sample solution with a fresh buffer solution. In an irreversible case, the complex-formation on the surface is expressed as follows:[4-6]

$$A_{fixed} + B \xrightarrow{k} C \qquad (1)$$

where A_{fixed} is an immobilized antibody on the surface; B, an antigen in solution; C, the complex formed on the surface, and k, the rate constant. On the assumption that the potentiometric response of the electrode, $U(t)$, is proportional to the surface concentration of C, [C], $U(t)$ is expressed as follows:

$$U(t) = U_m [1 - \exp(-k[B]_0 t)] \qquad (2)$$

where $[B]_0$ is the initial concentration of [B], t is the reaction time after the addition of B, and U_m is the response to be realized when all surface fixed anti-IgG molecules are complexed. In this equation, [B] is assumed to keep the initial value $[B]_0$, because the quantity of B in the solution is much larger than that of A_{fixed}. The obtained potential changes from Fig. 3 fit in well with Eq. 2, and by treating the data, $k=3.8\times10^4$ ($M^{-1}s^{-1}$) and $U_m=1.3$ mV (pH 8.6, 30°C) are derived. The k value is of the same order of magnitude as the $k=1.6\times10^4$ ($M^{-1}s^{-1}$) previously obtained for a titanium anti-hCG electrode to hCG.

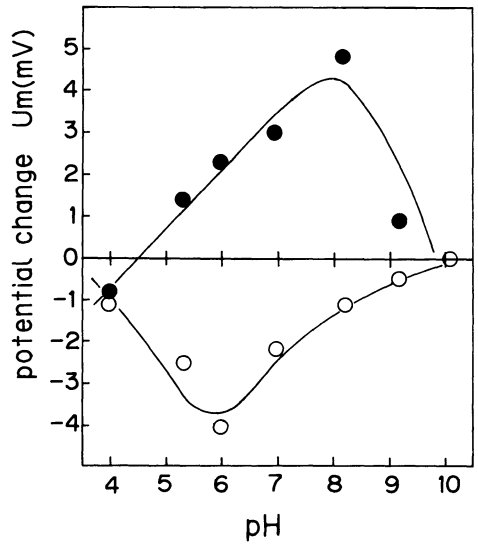

Fig. 4. The response of anti-IgG and IgG electrodes at various pH. ●: an anti-IgG electrode by an addition of IgG. ○: an IgG electrode by an addition of anti-IgG.

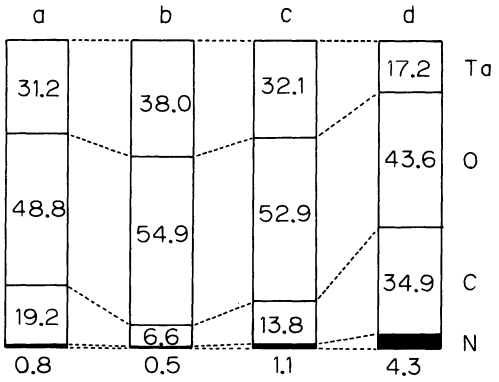

Fig. 5. The atomic concentration(%) of a tantalum surface measured by AES for each step of chemical modification. (a) After washing, (b) after O_2 plasma treatment, (c) after CNBr treatment, (d) after immersing in antibody soln.

The k values for reactions between various sets of antigens and antibodies, as determined by Karush, lie between 2×10^5 (M^{-1} s^{-1}) and 4.8×10^8 (M^{-1} s^{-1}).[14] Our present values are appreciably smaller than Karush's, like those obtained in our previous work.[4-6]

The antigen-antibody complex formed on the tantalum electrode can be dissociated by immersing it in a 2 M $MgCl_2$ stirred aqueous solution containing 1 M urea for 20—30 min. After this has been done, the anti-IgG electrode again shows a response to the antigen. Thus, the electrode could be used 7 to 8 times, although the response gradually became small; the potential could be restored by immersion in a fresh buffer. This degradation indicates a denaturation of the antibody by urea. In the case of IgG electrodes, the potential change upon the addition of anti-IgG-Fc is of almost the same order of magnitude as in the case of the anti-IgG-Fc electrode upon the addition of IgG except that the direction is negative.

pH Effect. The U_m values for the anti-IgG electrode in the reaction with IgG were determined from the data of the potential shift and Eq. 2 at various pH's. The U_m values thus obtained are always positive, from pH=5 to 9.2 (Fig. 4). The negative value at pH4 might have been caused by the partial denaturation of the protein, and so this value can be omitted. On the other hand, for the IgG electrode, the U_m value is always negative, from pH=4 to pH=9.2, with the minimum at pH=6. These results (shown in Fig. 4) indicate that the direction of the potential shift in the anti-IgG electrode is generally the opposite of that in the IgG electrode. As can be seen from Eq. 2, the U_m value is independent of the kinetics of the reaction or the binding energy between the proteins. It should be dependent on (1) the electrical dipole moment or the charge polarization between the antigen and antibody and (2) the coverage of the protein chemically attached to the electrode surface, because the change of the surface potential is linearly dependent on the electrical double layer at the interface, that is, the polarization per unit of surface area. Therefore, the results shown in Fig. 4 indicate that the direction of the electrical polarization between complexed protein molecules is independent of the pH. This implies that the polarization is mainly caused by a weak chemical bonding (a sort of charge transfer complexation or hydrogen bonding) between antigen and antibody. If, on the other hand, we assume that the potential shift is caused by the net charge of the protein molecule to be detected, its direction must be reversed at its isoelectric point (pH=5.8—7.3). The results in Fig. 4 clearly show this is not the case.

Surface Analysis. Figure 5 shows the relative atomic concentrations of the electrode surface at the successive steps of the chemical modification, calculated from the AES peak to peak heights for Ta(174 eV), C(273 eV), N(384 eV), and O(510 eV).[15] As the surface concentrations of N and C decrease from (a) to (b), one can see that organic contaminations are taken away by O_2 plasma treatment. The increase in the concentrations of N and C from (b) to (c) shows the attachment of an organic group such as imidocarbonate by BrCN. The incerase in the concentrations of N and C from (c) to (d) shows the attachment of a significant amount of anti-IgG to the surface. These results confirm each of the steps of the chemical treatment and support the idea that the result obtained from the potentiometric measurement is really caused by the specific complexing of immuno-proteins.

In conclusion, the specific detection of immuno-active proteins , IgG or anti-IgG, can be performed by the use of a tantalum electrode modified with the

antibody or antigen. The steps of the protein immobilization of the tantalum surface have been confirmed by AES.

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