Letters to the Editor

A Potentiometric Immunoglobulin G Sensor Based on a Polypyrrole Modified Platinum Electrode

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As electrochemical immunosensors are expected to provide simple devices for immunoassay, many efforts[1] have been devoted so far to develop them: Janata[2] prepared an immunoelectrode, on which Concanavalin A was covalently attached through coated poly(vinyl chloride) film. Aizawa *et al.*[3] developed immunosensors based on the transmembrane potential changes associated with immunochemical reactions. Ti electrodes chemically modified with antigen or antibody were applied to hormone analyses.[4] More recently, Rechnitz *et al.*[5] reported antigen-ionophore based membrane electrodes for digoxin antibody measurements. Furthermore, to increase the sensitivity, several so-called chemical amplification methods have also been developed.[6-9]

Although direct potentiometric immunosensors have such advantages as simple-handling and easy-reduction in size, those developed so far still have problems on slow response and/or low sensitivity; immunosensors are most behind in the field of biosensors. In the present study, we have demonstrated that a polypyrrole modified platinum electrode, on which the human immunoglobulin G (IgG) was immobilized, acts as an effective immunosensor for anti-IgG (from goat) of a new direct potentiometric-type.

Experimental

Polypyrrole modified platinum electrode (ϕ=0.5, l=5 mm) was obtained by electro-polymerization of pyrrole in an acetonitrile solution containing 0.1 M pyrrole, 10% glutaraldehyde (GA) (from a 50% aqueous solution) and 0.1 M $(C_4H_9)_4NBF_4$ as a supporting electrolyte at a constant current of 5 μA for 3 hours; IR spectra of the film formed showed that GA was incorporated in polypyrrole film, but the reaction of GA with >NH of pyrrole was less. IgG was then immobilized by dipping the polypyrrole modified electrode at 4°C for overnight into a phosphate buffer (PB) solution (pH 7) containing human IgG (150 mg/ml) by use of the Schiff base formation between -CHO of GA incorporated in the polypyrrole film and $-NH_2$ of IgG. The electrode was then treated with a PB solution of 0.1 M $NaBH_4$. Induced potential changes of the IgG immobilized electrode, thus prepared, were measured with respect to an SCE using an electrometer in a PB solution without deaeration in the presence of various amounts of anti-IgG under stirring.

Results and Discussion

The IgG immobilized electrode showed no further response against IgG, but by adding anti-IgG, even at a very low concentration, the electrode potential shifted rapidly toward negative direction; typical response curves are shown in Fig. 1. Such rapid response and high sensitivity did not obtained when IgG was adsorbed directly onto a bare Pt electrode or a polypyrrole (without incorporated GA) modified electrode, indicating that GA introduced the sites at which IgG linked covalently on the modified electrode surface in large amounts (*ca.* 10^{-7} mg/cm^2). The present electrode did not respond against some proteins tested, such as hemoglobin, myoglobin, ovalbumin and so on.

The induced changes in potential observed are much larger than those reported previously.[1-5] Thus, a different origin for the potential change from those of other immunosensors are expected. For the present potential change induced, dissolved oxygen in the test solution plays an important role; under an N_2 atmosphere, no significant negative shift in potential was observed. Neither the membrane potential change nor the negative charge accumulation at the electrode surface (IgG as well as anti-IgG used is negatively charged at pH 7) induced by the immunochemical reaction, can explain the effect of O_2 on the observed

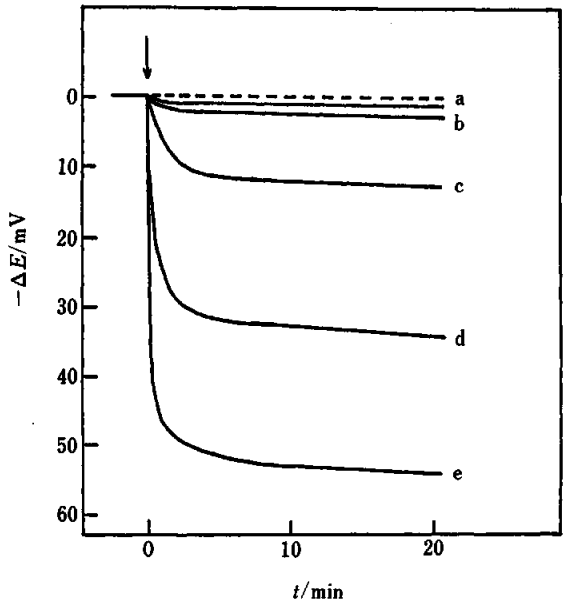

Fig. 1 Induced potential change (ΔE) with time of the IgG immobilized polypyrrole modified electrode by adding various amounts of anti-IgG: (a) 4.76×10^{-4}, (b) 4.76×10^{-3}, (c) 4.76×10^{-2}, (d) 4.76×10^{-1} and (e) 4.76 μg/ml. ---, 3.57 μg/ml of IgG.

potential change. A possible origin is as follows: The immuno-complex formed lowered the permeability of dissolved O_2 and the O_2 concentration at the electrode surface decreased, resulting in the negative shift in potential; the electrode potential would be consisted of some equilibrium potentials concerning dissolved O_2, such as O_2/H_2O_2 and O_2/H_2O. When a potential determinant, *e.g.*, ferri-cyanide ion, is in the solution, similar potential change to that in Fig. 1 was observed, even under an N_2 atmosphere; in this case, decrease in the permeability of ferri-cyanide ion through the immuno-complex layer would cause the potential change. Thus, for the present immunosensor, no Nernstian relationship between the induced potential change and an immunological component in the solution (anti-IgG, in this case) is expected. However, the observed response and calibration curves were rather reproducible (within ±5%) for the electrodes prepared under the same conditions. Also, the semiconductive film provides a low-impedance measuring system, and thus a high S/N ratio is easily obtained.

The potential change observed is clearly attributable to an initial immunochemical reaction, which itself is rather rapid; without stirring the solution, rapid response was again observed, but the potentials became less stable. The present anti-IgG sensor gave high sensitivity with rather wide measurable range (*ca.* 10 ng/ml to *ca.* 5 μg/ml or more), as shown in Fig. 2, and rapid response (*ca.* 2 min for 90% response, see Fig. 1); to our knowledge, the present new type sensor would be the most effective among direct potentiometric immunosensors developed to date, as far as the sensitivity (the detection limit was *ca.* 10^{-10} M or 10 ng/ml of anti-IgG) and response time are concerned. Furthermore, although the present results are still primitive, use of electro-polymerized semiconductive films seems to be promising for developing a new field of direct potentiometric immunosensors.

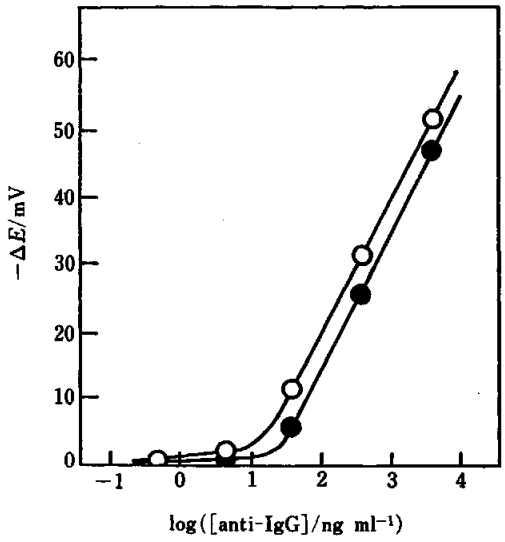

Fig. 2 Calibration curves of the IgG immobilized electrode for anti-IgG. ΔE values plotted were those obtained at 2 (●) and 10 (○) min after adding anti-IgG.

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References

1. F. W. Scheller, F. Schubert, R. Renneberg, H.-G. Muller, M. Janchen and H. Weise, *Biosensors*, **1**, 135 (1985).
2. J. Janata, *J. Am. Chem. Soc.*, **97**, 2914 (1975).
3. M. Aizawa, S. Suzuki, Y. Nagamura, R. Shinohara and I. Ishiguro, *J. Solid-Phase Biochem.*, **4**, 25 (1979).
4. N. Yamamoto, S. Shuto and H. Tsubomura, *Nippon Kagaku Kaishi*, **1980**, 1562.
5. M. Y. Keating and G. A. Rechnitz, *Anal. Chem.*, **56**, 801 (1984).
6. M. Aizawa, A. Morioka and S. Suzuki, *J. Membr. Sci.*, **4**, 221 (1978).
7. K. Kano, T. Ikeda and M. Senda, *Agric. Biol. Chem.* [Tokyo], **47**, 1043 (1982).
8. K. Shiba, Y. Umezawa, T. Watanabe, S. Ogawa and S. Fujiwara, *Anal. Chem.*, **52**, 1610 (1980).
9. P. W. Alexander and C. Maltra, *Anal. Chem.*, **54**, 68 (1982).

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