Biomembrane-mimetic Ion Sensors based on Multiphase Material containing Synthetic Polypeptide as Sensitizer

Mizuo Maeda,* Yasunori Tsuzaki, Koji Nakano and Makoto Takagi*

Department of Organic Synthesis, Faculty of Engineering, Kyushu University, 6-10-1, Hakozaki, Higashi-ku, Fukuoka 812, Japan

A biomembrane-mimetic sensor with an unusual stability was prepared from a multiphase material, *i.e.* poly(styrene-coacrylonitrile)-poly(L-glutamate) block copolymer **1**, which was coated on a Pt wire; the sensor showed electrochemical response towards Ca^{2+} based on the ion-induced conformational transition of the polypeptide as sensitizer.

One promising way to develop new principles in chemical sensor technology may be to study the unique features of biological sensing systems. In this context, the concept of 'ion-channel sensors' developed by Umezawa *et al.*[1] is of great interest since some of the sophisticated mechanisms occurring in biological system are successfully mimicked by using Langmuir–Blodgett (LB) membranes coated on a solid electrode. The LB membranes, however, appear not to be very stable on the electrode when exposed to aqueous media. In addition, the LB membranes may not be a good model for biological sensing since lipids in biomembrane mainly form a permeation barrier and thereby establish cell compartments, whereas membrane-bound macromolecules are responsible for most of the dynamic processes including ion-sensing and -channelling.

We describe in this communication a newly constructed biomembrane-mimetic sensor: a stable polymer membrane bearing synthetic polypeptide as a sensitising macromolecule was coated on a Pt wire. Electrochemical response towards Ca^{2+} was obtained based on the permeability change of the redox-active couple ions.

The design of the Ca^{2+}-receptive polymer membrane (Fig. 1) builds on our previous work on the divalent cation-induced permeability control of multiphase polymer membranes containing poly(L-glutamic acid) chains.[2] Poly(styrene-co-acrylonitrile) (M_n = 6000, styrene : acrylonitrile = 5 : 1) having a terminal carboxy group, which was kindly supplied by Toagosei Industry Co., Ltd., was treated successively with thionyl chloride and ethylenediamine to give the copolymer terminated with a primary amino group. The primary amino group-initiated polymerization of γ-methyl L-glutamate *N*-carboxyanhydride (a gift from Ajinomoto Co., Ltd.) gave a block copolymer; poly(styrene-co-acrylonitrile)-b-poly(γ-methyl-L-glutamate) **1**. Number averaged degree of polymerization (DPn) of the polypeptide segment was 30 as estimated by ^1H NMR spectroscopy. A Pt wire (diameter, 0.5 mm) sealed into the end of a glass tube (diameter, 5 mm) to leave 1 cm of the wire exposed was dipped into a 1,2-dichloroethane solution of **1** (1 wt-%) and dried to give the polymer-modified electrode.

Cyclic voltammetric measurements were performed by using a Solartron Co. Model 1286 potentiostat with a conventional design of a three-electrodes system. A Pt wire

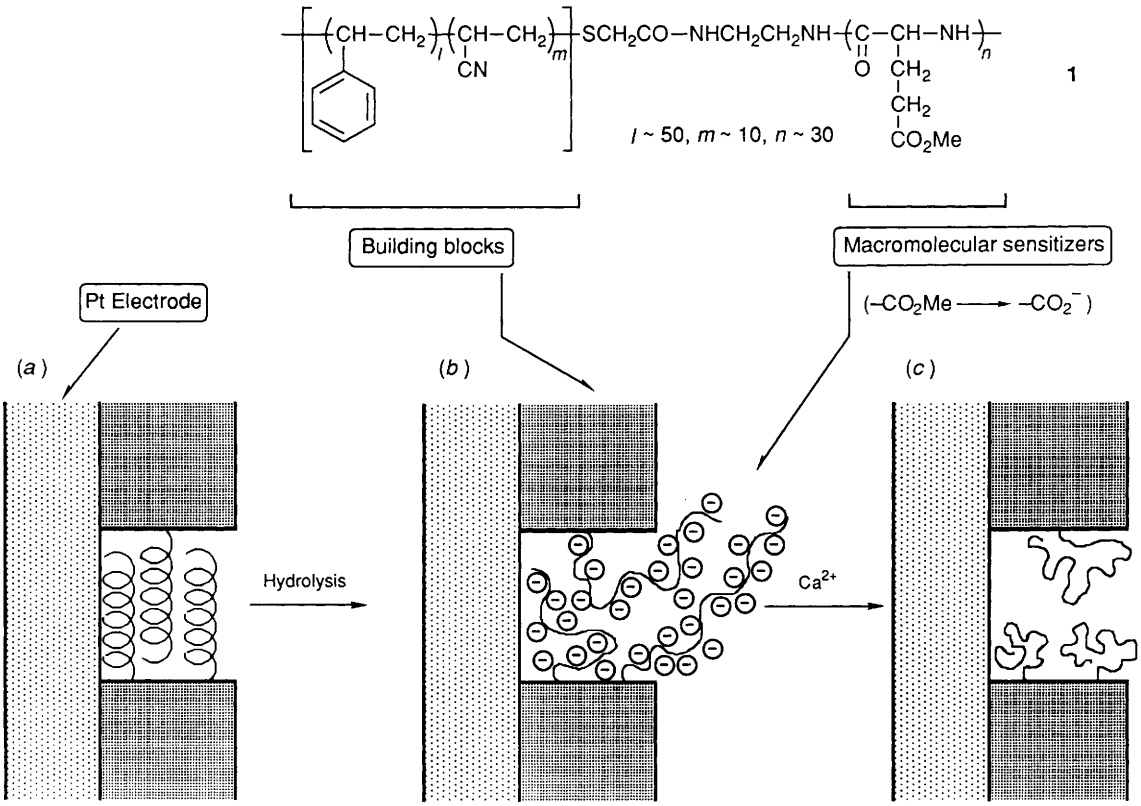

Fig. 1 Schematic illustration of the biomembrane-mimetic sensor and its operation.

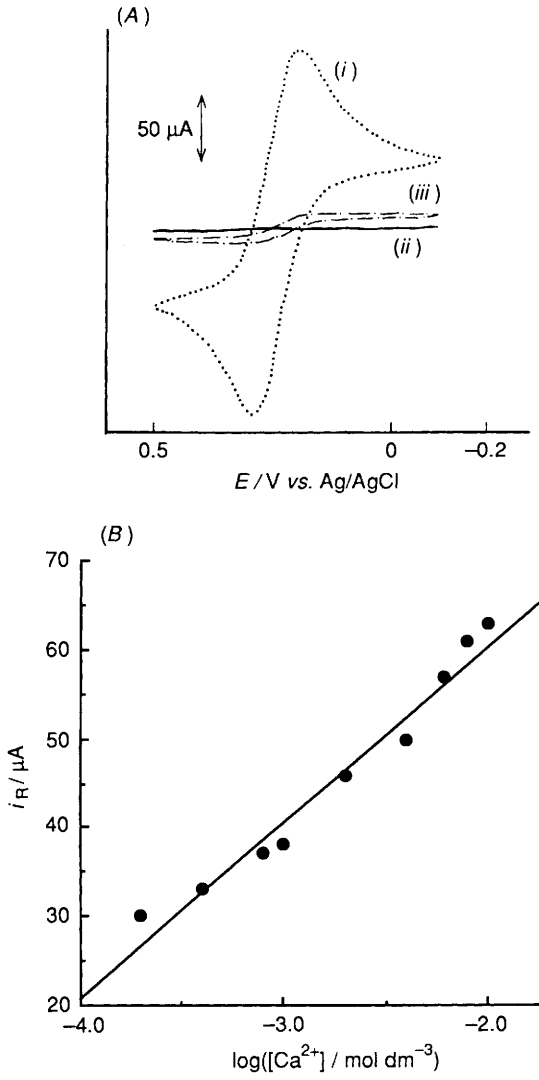

Fig. 2 Cyclic voltammetric responses of a Pt electrode modified with **1** at room temperature; scan rate, 25 mV s^{-1}; $[K_4[Fe(CN)_6]] = [K_3[Fe(CN)_6]] = 5$ mmol dm^{-3}, [KCl] = 0.1 mol dm^{-3}: (*A*) cyclic voltammograms for (*i*) bare Pt electrode, (*ii*) Pt electrode modified with **1** and (*iii*) modified electrode after hydrolysis; (*B*) Ca^{2+}-dependent changes in the reduction peak current (i_R) at around 0.14 V (*vs.* Ag/AgCl).

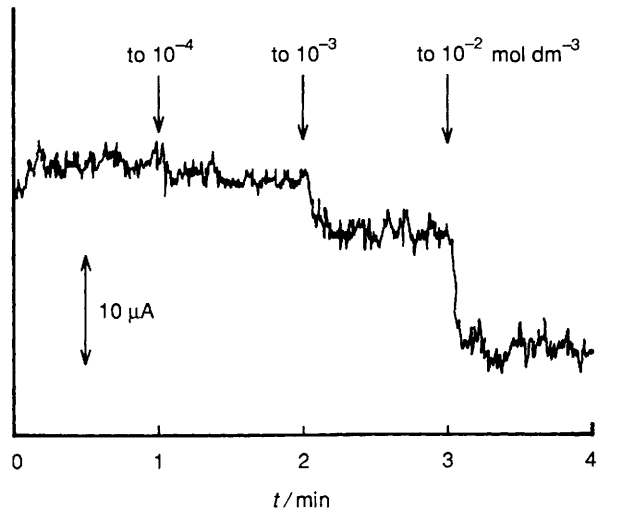

Fig. 3 Amperometric response towards Ca^{2+} of the polymer modified electrode at room temperature. The electrode potential was fixed at 0.4 V (*vs.* Ag/AgCl); $[K_4[Fe(CN)_6]] = [K_3[Fe(CN)_6]] = 1$ mmol dm^{-3}, [KCl] = 0.1 mol dm^{-3}.

and a standard Ag/AgCl (saturated KCl) electrode were used as counter and reference electrodes, respectively. Cyclic voltammograms of the ferrocyanide/ferricyanide redox couple with the modified electrode are shown in Fig. 2. The peak currents due to the reversible electrode reaction of a $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ system on a bare Pt electrode were almost completely suppressed by the modification with **1** (Fig. 2*A*). When the modified electrode was treated for 12 h with a ternary solvent of water, methanol and propan-2-ol (1 : 2 : 2, v/v/v) containing 0.5 wt-% KOH in order to hydrolyse the methyl ester group of the polypeptide to the carboxylate group, the CV peaks reappeared at almost the same potential as those on the bare electrode, but with much smaller current values. On addition of Ca^{2+} ions (as $CaCl_2$), the peak currents increased significantly with increasing concentration of Ca^{2+}. The current value for the reduction peaks around 0.14 V (*vs.* Ag/AgCl) showed almost an linear relationship with log $[Ca^{2+}]$ in the range 10^{-4}–10^{-2} mol dm^{-3} (Fig. 2*B*; correlation coefficient, 0.983; $n = 9$). The oxidation peaks around 0.3 V could also be used to prepare a calibration curve. Such ion concentration-dependent changes in CV profiles were also seen for Mg^{2+}, but not for Na^+, K^+ and tetrabutylammonium ions.

The behaviour is fully reversible; the same CV curve as the starting one reappeared when the electrode was immersed in 0.1 mol dm^{-3} KCl for a few minutes. The modified electrode has a remarkable stability; it responded reproducibly to Ca^{2+} for more than two months, when stored in 0.1 mol dm^{-3} KCl at room temperature. Amperometric detection of Ca^{2+} using the same electrode was also possible (Fig. 3). A rapid response within several seconds was obtained. The response contained a relatively large noise component, which may be reduced by refining the measurement system, *e.g.* electrode configuration, cell geometry and shielding.

These results may be explained as follows: the electrode is first covered with the hydrophobic polymer **1** (Fig. 1*a*) and is insulated from the redox-active species. Then, on hydrolysis, the polypeptide segment of **1** is converted to poly(L-glutamate) which forms hydrophilic microdomains (Fig. 1*b*). The microdomains containing flexible polyanionic chains function as 'ion channels' for the redox species so that the CV peaks appear. The electrode surface is, however, still covered to some extent with hydrophobic vinyl polymer segments which serve as the building blocks of the membrane. In addition, the polyanion network would reduce the local concentration of the anionic redox couple near the electrode surface, mainly due to electrostatic repulsion. With the addition of Ca^{2+} ionic crosslinking of carboxylate groups should occur, resulting in a contracted conformation of poly(L-glutamate) chains (Fig. 1*c*). The relatively 'open' feature of the 'ion channels' as well as the reduced number of anionic sites in the channel is considered to account for the increase in the local concentration of the redox species, leading to the enhancement of the peak currents. Selectivity among divalent ions would not be expected since the mechanism depends simply upon the ionic crosslinking of the polyelectrolyte, so that some additional

modification of the sensitizer is required for further specificity.

It is known that a conformational change of polypeptides takes place on applying various stimuli including H^+, ions, chemical substances, temperature change, solvent composition change *etc.* By adopting some of these changes, multiphase polymer membranes containing polypeptide have been designed[3] and shown to respond to pH,[4] detergents[5] and urea[6] with modification of membrane permeability. The present polypeptide-containing copolymers should serve as a new type of key material for chemical modification of electrodes.

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