Non-enzymatic Response Towards Urea using a Poly(L-glutamate)-modified Pt Electrode

Mizuo Maeda,* Yuji Fujita, Koji Nakano and Makoto Takagi*

Department of Chemical Science and Technology, Faculty of Engineering, Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812, Japan

An electrochemical response towards urea was obtained by using a poly(L-glutamate)-immobilized Pt electrode which does not comprise the enzyme-relying transducer.

One of the successful examples of enzyme electrodes may be a urea sensor which contains urease as a sensitizer immobilized on ion-selective electrodes. In addition to the role in highly-specific chemoreceptive processes, the enzyme has another important task to convert the analyte to the electrochemically detectable species such as ammonia and carbon dioxide.[1] Thus, the urea electrode comprises the enzyme-relying transducer for obtaining electrochemical responses.

Recently, Sugawara and coworkers have presented the concept of 'ion-channel sensors', the most important features of which involve an electrochemical response towards uncharged, non-dissociable and/or redox-inactive molecules.[2]

We describe in this communication an enzyme-free electrode responsive to urea. A sensitizer used was a synthetic polypeptide, poly(α-L-glutamate) (PLG), which undergoes conformational changes depending on the concentration of urea.[3] PLG was easily immobilized on a Pt wire with the aid of multiphase polymer material, poly(styrene-co-acrylonitrile)-PLG block copolymer.[4] Electrochemical response towards urea was obtained based on the permeability change of the redox-active couple ions (the 'ion-channel' mechanism[2]).

Preparation of the modified platinum electrode was described previously.[4] The electrode has surface-immobilized PLG chains which form hydrophilic microdomains as 'channels' for the redox species, whereas the platinum surface is covered to some extent with hydrophobic vinyl polymer segments which serve as the anchors for the modifying layer (Fig. 1).

Cyclic voltammetric (CV) measurements were performed by using a Solartron Co. Model 1286 potentiostat with a conventional design of a three-electrodes system. A Pt wire and a standard Ag/AgCl (saturated KCl) electrode were used as counter and reference electrodes, respectively. Cyclic voltammograms of ferrocyanide/ferricyanide redox couple with the modified electrode are shown in Fig. 2(*a*). The peaks due to the reversible electrode reaction of a $Fe(CN)_6^{4-}$/$Fe(CN)_6^{3-}$ system appeared at almost the same potential as those on the bare Pt electrode, though the current values were

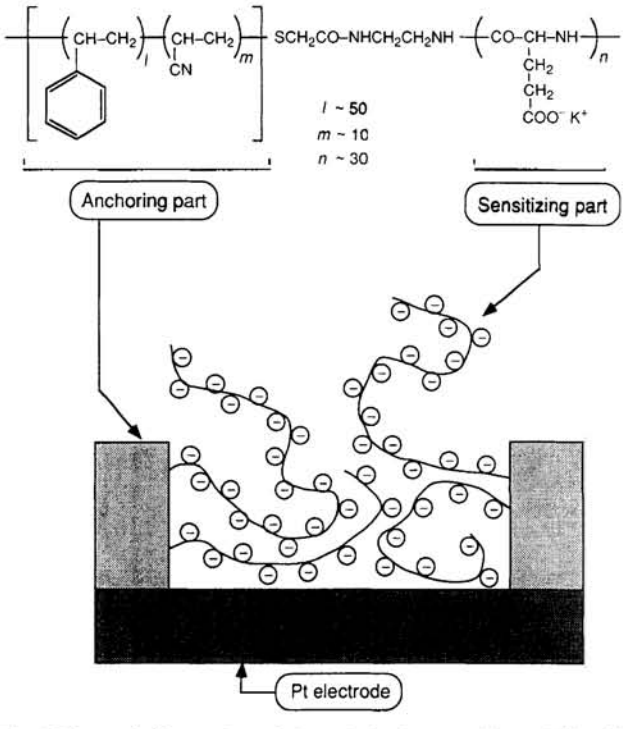

Fig. 1 Schematic illustrations of the poly(L-glutamate)-immobilized Pt electrode

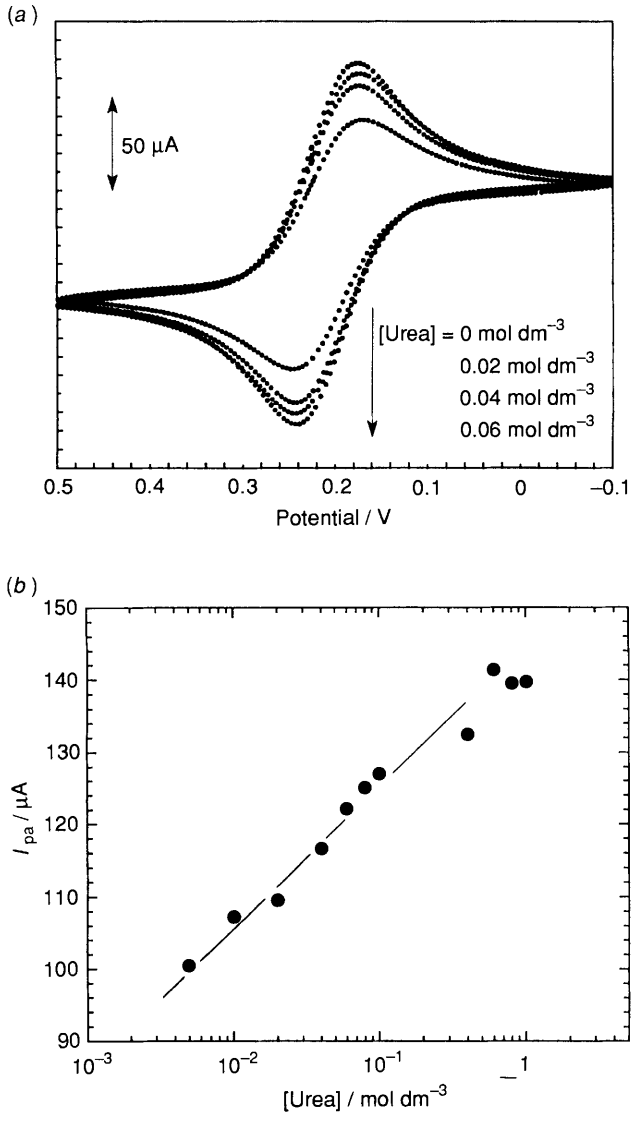

Fig. 2 Cyclic voltammetric responses of the poly(L-glutamate)-modified Pt electrode at room temperature; scan rate, 25 mV s^{-1}; [K_4[Fe(CN)$_6$]] = [K_3[Fe(CN)$_6$]] = 5 mmol dm^{-3}, [KCl] = 0.1 mol dm^{-3}: (*a*) the urea-dependent change in CV profiles; (*b*) [urea] *vs.* anodic peak current (I_{pa})

very small (*ca.* 50% of the bare one). When adding aqueous urea, however, the peak currents increased significantly with increasing concentration of urea as seen in Fig. 2(*a*). The anodic peak current (I_{pa}) showed almost a linear relationship with the logarithmic concentration of urea in the range of 5 × 10^{-3}–6 × 10^{-1} mol dm^{-3} [Fig. 2(*b*); correlation coefficient, 0.987; n = 9]. The dynamic range bears comparison with the conventional enzyme electrodes, as discussed later. Such urea-dependent changes in CV profiles were not seen at all on a bare Pt electrode within this concentration range.

Urea is widely known as a denaturant for proteins. The denaturation process has been considered to be due to the breaking of hydrogen bonds and of hydrophobic bonds,[3] resulting in the conformational change from ordered to random-coil form. Urea exerts its denaturing action also on synthetic polypeptides including poly(L-glutamic acid).[3,5] The effect has been utilized to regulate the permeability of a polymer membrane incorporating unionized poly(L-glutamic acid) chains.[6] The present strategy to detect urea non-enzymatically has been built upon this previous success.

The urea-induced enhancement observed here of the peak current can be explained by the permeability change of the redox species through the PLG-modified layers. However, the mechanism for the permeability change would not be ascribable simply to the conformation of the electrode-bound PLG chains as in the previous work,[6] since the polypeptides were believed to be in the fully ionized, randomly coiled state through the present measurements.[4] In fact, the intrinsic viscosity of PLG random coils was reported to be insensitive to urea at ionic strength of 0.1 mol dm^{-3}.[5] Thus, the drastic change observed in the present CV profiles is rather surprising.

An explanation may be that a strong, attractive interaction of urea with the peptide groups (and/or the side chain carboxy groups) would crosslink PLG chains to be contracted, resulting in the relatively 'open' feature of the 'ion channel'. Although the putative favourable interaction of urea with those groups has been found too small, if any, to induce conformational changes of fully-ionized PLG in aqueous solution,[5] the PLG chains in the present case are densely anchored on the electrode and are surrounded by the matrix from the hydrophobic vinyl polymer chains: the electrode surface bristling with PLG chains could be a peculiar environment for the action of urea as well as for the electrochemical reaction of ferrocyanide/ferricyanide redox couple. Another well-demonstrated effect of urea on PLG is to raise the pK_a of the side chain carboxy groups.[3] If this is the case on the electrode, the reduced number of anionic sites brought about by the addition of urea would account for the increase in the local concentration of the anionic redox species, leading to the enhancement of the peak currents.

A urea sensor has conventionally consisted of a urease-immobilized membrane and an electrode for the determination of the metabolites; electrodes for ammonium ion,[7] pH,[8] ammonia gas[9] and carbon dioxide[10] have been used. These sensors were all potentiometric ones to give Nernstian responses, the linear concentration range of which was typically between 10^{-4} and 10^{-1} mol dm^{-3} of urea. If one wishes to detect urea with using the ion- or gas-selective electrodes, urease is indispensable not only for specific recognition based on the bioaffinity interaction but also for chemical conversion of urea to detectable species, since urea is essentially electro-inactive. On the contrary, the polymer-modified electrode described here does not rely upon enzymes; the concentration of urea can be determined directly.

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