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Biomimetic sensors using the 'gate effect' of molecularly imprinted polymers

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Abstract

Molecularly imprinted polymers (MIPs) are recognition materials that can be prepared by a tailor-made process. The function of MIPs is similar to that of antibodies, but is much more robust and economical. Thus, MIPs have been expected as recognition elements in chemical sensors; however, this application has yet to be achieved. The permeability of some types of MIPs depends on specific interactions with their templates. This phenomenon is termed the "gate effect." The gate effect of MIPs grafted onto an electrode can be detected by amperometric methods. The sensing method is very simple, selective, and highly sensitive, and thus is feasible for an extreme number of applications.

Key words: molecularly imprinted polymer (MIP), gate effect, faradic current, and permeability

Introduction

L. C. Clark first published the concept of biosensors, in which an enzymatic reaction is transduced into electric signals [1]. He invented a glucose sensor using glucose oxidase immobilized on an oxygen electrode. Biosensors realize high selectivity using molecular recognition of biopolymers (e.g., enzymes or antibodies). In particular, they are useful for detecting a specified chemical in sample fluids containing many types of impurities, such as blood and fermentation fluids. However, enzymes and antibodies are not chemically or physically robust and cannot endure harsh conditions. In his original paper [1], Clark proposed sensors that could be implanted into patients' bodies in order to monitor the chemical composition of body fluids. However such applications have not been realized, largely due to the lack of robustness of the biopolymers. They cannot endure the sterilization procedures (e.g., using an autoclave or γ-ray irradiation) that are essential for implantable devices. Chemical modification to improve biocompatibility is also required; however, biopolymers cannot endure these operations either. Therefore, substitutes for biopolymers are needed for implantable sensors. Synthetic materials that have molecular recognition ability would be promising as substitutes. Molecularly imprinted polymers (MIPs) have been the focus of our attention.

MIPs are prepared by template polymerization in order to create a polymer with the ability to bind specifically with the template. A typical process for the preparation of an MIP is as follows: (i) a target molecule or template is allowed to conjugate with a functional monomer via non-covalent or weak covalent binding; (ii) the functional monomer is allowed to copolymerize with a crosslinking monomer; and (iii) the template is removed from the copolymer (Fig. 1). As a result, the binding site for the template is imprinted in the matrix of the highly crosslinked copolymer. The MIP can be prepared by a tailor-made process, just as an antibody can. However, MIPs can be prepared much more cheaply, quickly and simply. In addition, MIPs are more robust than biopolymers. Thus, MIPs are expected to be useful as molecular recognition elements in chemical sensors. It should be possible to transduce the specific binding event into electric signals for this type of application. Transductions by quartz crystal microbalance (QCM) or surface plasmon resonance (SPR) have been attempted by many researchers. However, the templates are usually much smaller than the MIPs, and therefore the template in the MIPs is hard to detect by those methods. To address this issue, we have developed a new sensing method using the gate effect, which is an inherent property of MIPs.

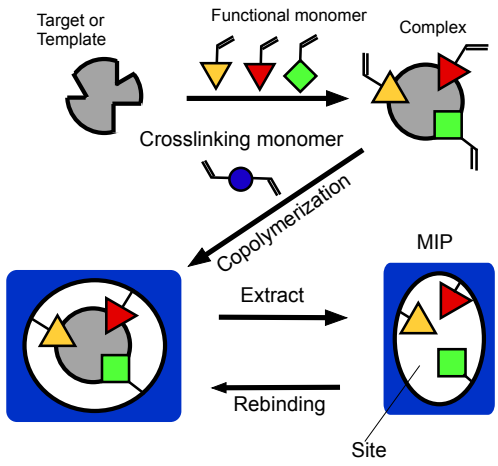

Fig. 1: Concept of molecularly imprinted polymers (MIPs).

What is the gate effect?

Gate effect is the change in the porosity and the permeability of MIP responding to the specific interaction with the template. In the “gate" analogy, the template corresponds to the “key", and the site in the MIP for specific rebinding with the template corresponds to the “keyhole".

Initially, a MIP was grafted to an indium tin oxide (ITO) electrode using theophylline as the template [2]. The anodic current of ferrocyanide at the MIP-grafted electrode was dramatically enhanced by the presence of the template. The surface morphology of the grafted electrode imaged using an atomic force microscope (AFM) was also dramatically changed by the presence of the template as shown in **Fig. 2.** The change in morphology possibly indicates a change in the porosity of the MIP layer. The change in faradic current can also be attributed to a change in the permeability and porosity of the MIP layer, as shown in Fig. 3.

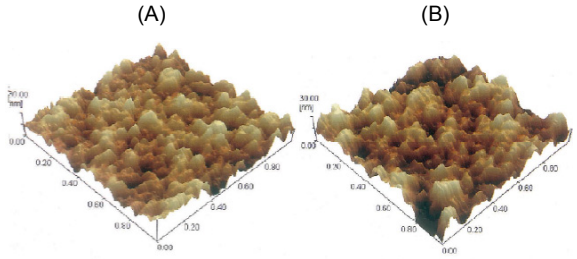

Fig. 2: Atomic force microgram of theophylline imprinted-electrode in water in the absence (A) or presence (B) of the template [2].

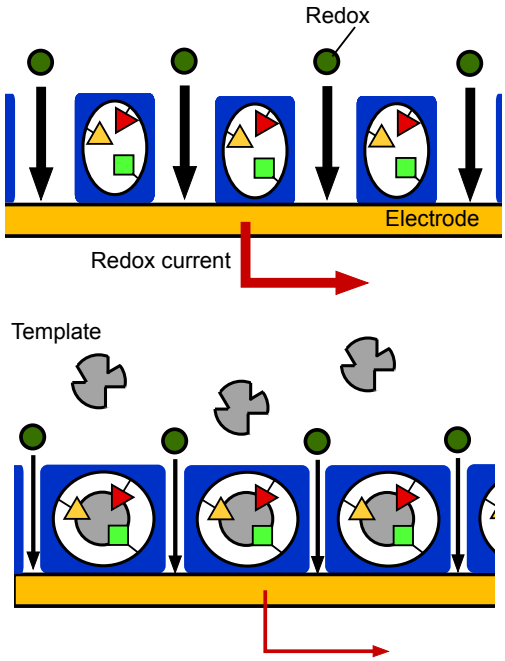

Fig. 3: Electrochemical sensing using the gate effect.

In order to prove these speculations, the theophylline-imprinted polymer was grafted onto a cellulosic semi-permeable membrane using the same procedure [3]. The permeability was dramatically increased by the presence of the template (theophylline), but was insensitive to the presence of an analogue of the template (caffeine). It was thus clarified that the grafted MIP-layer changes its permeability in response to the template.

Some researchers have noticed a change in the porosity of MIP membranes because of an interaction with the template. For example, S. Piletsky *et al*. discovered that the conductivity of an MIP membrane in the electrolyte solution is sensitive to the template [4, 5]. He speculated that the result is due to a change in the porosity of the MIP controlling the permeability of the ions.

Some researchers also reported that the morphology of mildly crosslinked molecularly imprinted polymer gels is sensitive to the template. M. Watanabe *et al.* reported that the volume of a molecularly imprinted *N*-isopropyl acrylamide gel in the shrunken state was sensitive to the presence of the template [6]. T. Miyata *et al.* reported that the volume of an antigen-imprinted acrylamide gel, in which the acrylated antibody was used as a functional monomer, was sensitive to the template [7].

The results of these investigations support the idea that the MIP changes its porosity because of a morphological change due to site-specific interaction with the template. It is natural to think that these phenomena are also related to the gate effect described above. However, the applicability for sensor development has not been sufficiently discussed.

The existence and potential value of the gate effect was further confirmed with the successful selective sensing of enantiomers by the MIP-grafted electrode [8]. The faradic current at the electrode imprinted by L- (or D-) phenylalanine was sensitive to the presence of the template, but insensitive to the other enantiomer, even though both optical isomers have the same chemical and physical properties. Therefore, the difference in the current must have been due to the site-specific binding capability, which was the result of the imprinting during the polymerization.

The detection of a faraday current is very simple, and the gate effect is feasible for sensing many types of chemicals with very high specificity. In addition, MIPs are chemically and physically more robust than antibodies or enzymes and can endure sterilization procedures. As a result, the use of MIP-grafted electrodes in clinical sensor applications is feasible. In particular, the electrode is promising as an implantable sensor for the monitoring of the chemical composition of body fluids.

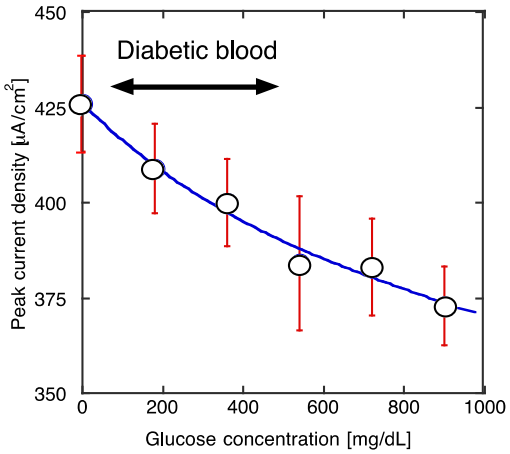

Fig. 4: Calibration curve of the glucose-imprinted electrode [9].

To demonstrate the potential of the new MIP-based electrode, a glucose sensor was designed using the gate effect of MIPs based on covalent binding. Glucose was allowed to bind with 4-vinylphenyl boronic acid (VPBA) [9]. The adduct of VPBA and glucose was copolymerized with methylene bisacrylamide, which is a crosslinking monomer that was then grafted to an ITO electrode. The glucose was extracted by washing with dilute acid. The oxidative current at the grafted electrode was sensitive to the glucose concentration (**Fig. 4**), and thus the covalent-type MIP exhibited the gate effect, and it was confirmed that a glucose sensor can be realized based on the gate effect.

The sensing of heparin in blood using the gate effect has also been successfully achieved [10]. The response time is around 15 s, which is much shorter than that of conventional monitoring methods for heparin in blood.

Mechanistic study of the gate effect

The mechanism of the gate effect is still a black box. However, an understanding of the mechanism is essential for optimization of its use in sensing applications. In particular, a comparison of the capacity and selectivity of the adsorption is essential. The MIP-layers grafted onto an electrode by our method are very thin (less than 10 nm). As a result, it is difficult to determine the amount of the template that is binding with the imprinted site of the MIP.

Therefore, a molecularly imprinted self-supporting membrane (MISSM) was developed in order to model the gate effect [11]. The MISSM was formed by photo-irradiation of a mixture of monomers, template, and initiator placed between a couple of quartz plates. Flexibility of the membrane is needed for the MISSM, in order to evaluate diffusive permeability by dialysis method. Thus, triethyleneglycol dimethacrylate was used as a flexible crosslinking monomer. It was allowed to copolymerize with the functional monomers in the presence of L- or D- phenylalanine as the template. A 50-μm-thick flexible membrane was formed, and the template was then extracted using a 50-wt% aqueous solution of methanol.

The template adsorbed onto the membrane remarkably well, but the enantiomer of the template did not. Furthermore, the permeability of the membrane was increased by the presence of the template, but was insensitive to the presence of the enantiomer. Thus, the membrane exhibited the gate effect. Even a small amount of adsorbed template (as low as 0.1 wt% of the MISSM membrane) resulted in a remarkable change in the permeability of up to several dozen percent [12]. This result shows that the gate effect can enable highly sensitive detection of templates that would be difficult to achieve using SPR or QCM.

Fig. 5 shows the relationship between the solution content, which corresponds to volumeric porosity, in the membranes and the methanol concentration in the solvent. The

solution content in the membrane increased with increasing methanol concentration, following a sigmoid curve. The solution content increased at high methanol concentrations, decreased at low methanol concentrations, and was insensitive to the methanol concentration at the inflection point. It is possible that some type of phase transition occurs at the inflection point (20 wt% methanol). This result indicates that the gate effect is not a result of the shift of the point of the phase transition by the template. In addition, the membrane is turbid at higher methanol concentrations and transparent at lower methanol concentrations. This result indicates that the heterogeneity of the membrane could be an important key factor for understanding the mechanism of the gate effect.

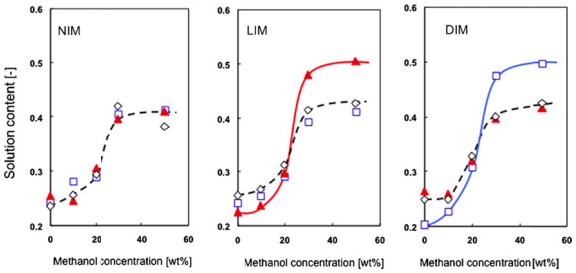

Fig. 5: A relationship between the solution content in the membrane and the methanol concentration in the solvent. (NIM: non-imprinted membrane; LIM: L-phenylalanine imprinted membrane; DIM: D-phenylalanine imprinted membrane; circle: 0 mM phenylalanine, triangle: 5 mM L-phenylalanine; square: 5 mM D-phenylalanine).

Conclusions

The gate effect associated with MIPs grafted onto electrodes is applicable for highly selective, sensitive and rapid-response biomimetic sensors. An MIP-grafted electrode can be prepared using a simple procedure and at low cost. The gate effect of an MIP on an electrode is detectable using a simple amperometric method, and thus this method is feasible for use in a wide number of applications.

The mechanism of the gate effect is, however, not yet understood. The MISSM is a potential tool for analysis of the mechanism. Investigation of the mechanism of the gate effect using the MISSM is underway in our laboratories.

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