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Potassium ion sensing using a self-assembled calix[4]crown monolayer by surface plasmon resonance

Hongxia Chen[a], Yeong-Soon Gal[b], Sung-Hoon Kim[c], Heung-Jin Choi[c], Min-Cheol Oh[d], Jaebeom Lee[a], Kwangnak Koh[a,*]

[a] *College of Nanoscience and Nanotechnology, Pusan National University, Pusan 609-735, Republic of Korea*
[b] *Chemistry Division, Kyungil University, Kyungsanbuk-do 712-701, Republic of Korea*
[c] *College of Engineering, Kyungpook National University, Taegu 702-701, Republic of Korea*
[d] *College of Engineering, Pusan National University, Pusan 609-735, Republic of Korea*

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ABSTRACT

The precise detection of K^+ ion is crucial because K^+ ion plays a leading role in membrane transport. Current K^+ ion detection methods suffered low resolution and detection limit. Calix[4]crown-5 derivatives are well known as K^+ ionophores. We described here a K^+ ion-sensing system using a self-assembled monolayer of calix[4]crown-5 derivative (calix[4]crown) modified gold chip based on surface plasmon resonance (SPR). The calix[4]crown sensing layer was characterized by atomic-force microscopy (AFM), SPR, Fourier transform infrared reflection absorption spectroscopy (FTIR-RAS) and cyclic voltammetry (CV). It was found calix[4]crown was assembled as a monolayer on Au surface. The SPR angle was found to be modulated by various concentrations of K^+ ion due to the interaction between the calix[4]crown and K^+ ion. This calix[4]crown monolayer showed a more sensitive and selective binding toward potassium ion over other alkali and alkaline earth metal ions. From the simple SPR spectroscopic analysis, we were able to monitor K^+ ion concentration with a wide range of 1.0×10^{-12} to 1.0×10^{-2} M in an aqueous solution with a pH 6–8. These experimental results showed a useful method for the design of simple and precise potassium ion biosensors.

1. Introduction

Biological active-transport systems involving ions, in particular K^+ ion, have important functions in the organism [1,2]. The K^+ ion concentration change in the plasma also results several diseases. Khander et al. found K^+ ion in the blood of different patients with cardiovascular diseases revealed quantity regularities that allowed the diseases to be diagnosed early [3]. The internal ionic environment of bacteria and eukaryotic cells is generally potassium-rich, leakage of K^+ ion has been used to monitor membranolytic events in erythrocytes, yeast cells, liposomes and bacteria [4–6]. These findings prompted studies on the potassium leakage mechanisms, regulation and energetic requirements of the efflux system. All of these studies need to measure efflux K^+ ion in a potassium-free medium from inner cell with a precise and continuous mode. Thus, several procedures are extensively employed for the K^+ ion determination, including spectrochemical methods, liquid chromatography and potentiometry. Additionally, fluorescent K^+ ion sensor and K^+ ion sensor based on capacitance measurement, which have a detection limit about 1 μM, suffer the imprecise for the detection of lower concentration of K^+ ion [7,8]. Currently, potentiometric lower detection limits are around 10 nM [9,10].

The surface plasmon resonance (SPR) sensor has been mainly used for the precise detection of biochemical interactions [11]. Recently, a novel molecular recognition system using a calixarene derivative has been applied to a SPR sensor [12–15]. Despite of the appearance of diverse recognition systems, there has no suitable recognition system established for sensitively sensing potassium ion using a SPR sensor, as it is not easy to obtain sufficient SPR response for the recognition of potassium ion. Calix[4]crown derivatives are well known as ion-sensing compounds. The ionophores based on calix[4]crown are very promising for practical sensing and separation. The selectivity depends on the length of the polyether chain and it shows a complementarity effect between the size of the cavity and the cation size [16–19]. Bochenska et al. reported the 1:1 of host–guest interaction between K^+ ion and calix[4]crown-5 derivatives. The NMR and

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* Corresponding author. Tel.: +82 55 350 5294; fax: +82 51 514 2358.
E-mail addresses: chenhongxia@pusan.ac.kr (H. Chen), koh@pusan.ac.kr (K. Koh).

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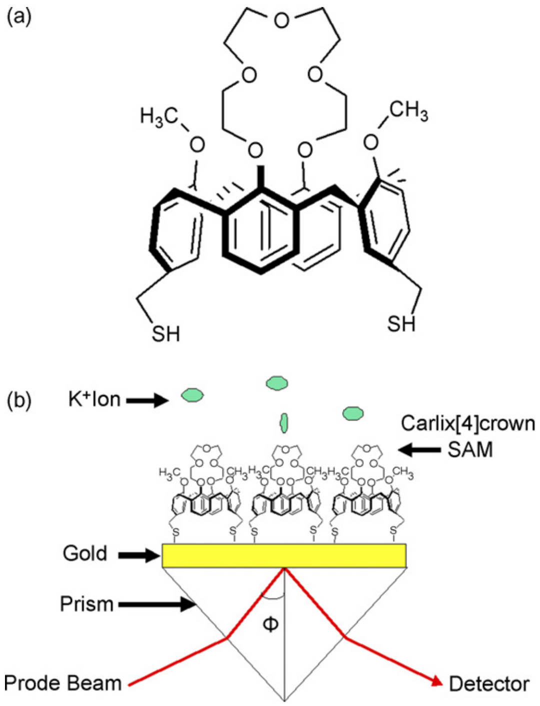

Fig. 1. (a) Molecular structure of calix[4]crown. (b) A sensor chip configuration.

X-ray structure showed K–O (crown) bond, K–O (low rim methoxy group) bound and K–p interactions in the complex, which are responsible for the selective and sensitive interaction with K^+ ion [20].

In this article, for the first time, in the best of our knowledge, we present the experimental results of a calix[4]crown-5 derivative (calix[4]crown) monolayer system to determine precise K^+ ion concentration. For the construction of well-characterized sensing layer, self-assembled monolayer (SAM) technique was applied to the organization of calix[4]crown molecules on the solid substrates surface. SAM technique guarantees each molecule to exhibit its original function on the surface [21]. The molecular recognition system generates a signal by specific molecular interaction between receptor and target molecules that was investigated by SPR. SPR is very sensitive to refractive index change of the interfacial surface and has been used to detect ions and small biomolecules coupled with self-assembled monolayers of complexing agents [22–24]. In our previous work, we had measured Ag^+ ion and monosaccharide at a concentration of 1×10^{-12} and 1×10^{-10} M, respectively [23,24]. This work provided a significantly useful method for the development of potassium ion sensing with high sensitivity and selectivity based on the coupling of SPR and SAM.

2. Experimental

2.1. Chemicals and reagents

Tris(hydroxymethyl)aminomethane, 1N hydrochloric acid, chloroform, methyl alcohol and all of the target metal ions with chloride salts were purchased from Aldrich Chemical Company (Milwaukee, WI, USA). Calix[4]crown (Fig. 1(a)) was obtained from Proteogen Co. (Seoul, Korea). A Milli-Q grade (>18.2 mQ cm) water was used for preparing the buffer solution. All of the metal ions solutions were made in a Tris–HCl buffer (pH 7.4, 0.10 M).

2.2. Formation and characterization of calix[4]crown SAM on the gold chip

A microscope cover glass (18 mm × 18 mm × 0.15 mm, refractive index = 1.515; Matsunami, Japan) with a gold layer was used as a substrate for the formation of calix[4]crown SAM. The gold film (thickness ≈50 nm) was deposited on the cover glass by the sputter coating system (Polaron Co., U.K.) under conditions of 2.0×10^{-2} mbar and 20 mA for 135 s. The sputtered Au substrate was rinsed using distilled water, methanol and acetone, sequentially. Then, the gold chip was dried in a nitrogen stream softly and ready to use.

The calix[4]crown solution was prepared by the mixture of chloroform and methanol. Their volume ratio was 1:100 (v/v). The calix[4]crown SAM was formed by immersion of the gold chip into the 0.10-mM calix[4]crown solution. The immobilization process was monitored by SPR spectroscopy. After the immobilization, the sensor chip was rinsed with chloroform–methanol mixture solution (1:100, v/v), methanol for 15 min and then dried under N_2 stream. The calix[4]crown SAM was carefully characterized by Fourier transform infrared reflection absorption spectroscopy (FTIR-RAS) and cyclic voltammetry (CV).

The FTIR-RAS spectra were measured with a resolution of $2\,cm^{-1}$. The glazing angle was maintained at 80. A p-polarized IR beam was used as the light source. For CV measurement, the electrode system consisted of an Ag/AgCl as a reference electrode, platinum coil as an auxiliary electrode and gold as a working electrode with a scan rate of 50 mV/s.

The SPR spectroscopic measurements for the monolayer formation of calix[4]crown and the interactions between the metal ions and the SAM were performed by a homemade SPR system based on the traditional Kretschmann configuration. A schematic diagram of the sensing scheme was shown in Fig. 1(b).

SPR spectroscopy recorded the buffer's saturation state as a base line. K^+ and other metal ions with seven different concentrations in a range of 1.0×10^{-14} to 1.0×10^{-2} M. We dilute the store solution of 10^{-2} M step by step using the precise syringe. The 100-μl sample solution was flowed into the Teflon SPR cell, following washed by buffer solution thoroughly to remove the physical adsorbed ions.

3. Results and discussion

3.1. Characterization of calix[4]crown SAM

To sense the formation of calix[4]crown SAM on the gold chip surface, SPR was measured regularly. As shown in Fig. 2(a), the SPR angle shifts were gradually increased and saturated inner 5 h. Also in Fig. 2(b), the typical FTIR-RAS spectra of calix[4]crown SAM on gold surface was confirmed. In particular, $\nu(sp^2$ C–O) stretching at 1203^{-1} confirmed the appearance of crown-ether group on the gold surface.

In some previous studies, the formation mechanism of alkanethiol monolayers on gold substrates has been proposed [25]. The properties of the electrode modified with calix[4]crown SAM can be estimated by submitting the electrode to reductive desorption experiments. Fig. 2(c) showed the reductive desorption peaks of calix[4]crown SAM on the gold electrode. This peak was attributed to the reductive desorption of thiolated compounds that was chemisorbed to the gold surface. Assuming that all thiolated compounds are reduced/oxidized in the CV experiments, the surface coverage can be determined from CV measurements [26]. After accounting for the surface roughness of the gold electrode,