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Impedance Characterization of Adsorption Process of Calmodulin on Au Substrate and its Combination with Ca^{2+}

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Abstract: In this paper, the adsorption process of calmodulin (CaM) on Au substrate was first investigated with electrochemical impedance spectroscopy (EIS) method. The result reveals that the adsorption of the protein-calmodulin contains two steps, *i.e.*, one short quick step followed by a slow one. The complexation of calmodulin with Ca^{2+} was also first probed using EIS technique, in which the complexation of CaM with Ca^{2+} could be reflected by the change of apparent membrane capacitance(C_{app}) clearly. In all above measurements, a redox couple $Fe(CN)_6^{3-}$/ $Fe(CN)_6^{4-}$ was used as probing-pin to reflect all the changes occurring in the above process. Our work suggests that some biological processes of CaM could be studied using EIS method conveniently.

Keywords: Calmodulin (CaM), adsorption behavior, Au substrate, complexation, electrochemical impedance spectroscopy.

Recently, the adsorption behavior of protein on solid substrate has attracted much attention for the reason that adsorption on a substrate is the first key step for other further electrochemical investigation[1]. Meanwhile the free adsorption could provide a relative well-ordered structure, with which some fundamental study could be carried out conveniently[2]. As we know, the electrochemical impedance spectroscopy (EIS) method has become one of the powerful techniques to detect the change of interfacial structure and the complexing process[3]. In addition, it was also reported that the complexation of ions with one specific fabricated membrane could be probed with EIS method with ease[3].

CaM is a ubiquitous Ca^{2+} binding protein of 148 residues. In addition, CaM plays a major role in a wide range of cellular Ca^{2+}-dependent signaling pathway[4]. CaM has been reported in many papers, but most reports only covered its structure[4]. To the best of our knowledge, there is no paper considering the adsorption process of protein, particularly for CaM with EIS technique. And reporting on the complexation of CaM with Ca^{2+} using EIS method was not found either.

The direct purpose of our work is to describe the adsorption process of CaM on Au substrate, and CaM complexation with Ca^{2+} as well with EIS method. In the case of its complexation with Ca^{2+}, the capacitance value obtained from EIS measurement suggested that the whole complexation process of CaM with Ca^{2+} could be divided into

three stages. The main contribution of our work is to provide one electrochemical method to reflect the behavior of CaM in the real biological system indirectly.

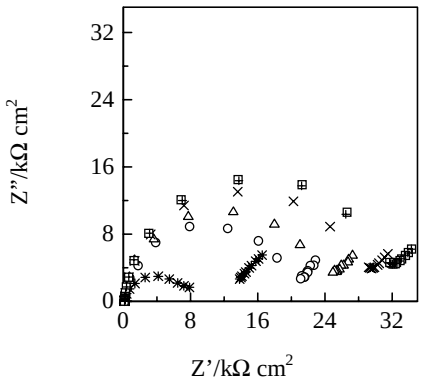
CaM was offered from the biological department of Hebei Teacher's University. The gold substrate was cleaned according to the previous report[5]. The treated Au substrate was transferred into an aqueous solution containing 10^{-5} mol/L CaM, and immersed in it for at least 24 h to complete its adsorption process. *Prior to* the experiment, the modified electrode (CaM/Au) was rinsed with water for 2 times, each time for 10 s. EIS experiment instrument has been described in the former report[5]. All the electrochemical experiments were performed in a traditional three-electrode system at room temperature. Saturated calomel electrode (SCE) and a large Pt foil were used as reference and counter electrode, respectively. All potentials reported here were respected to SCE. The complexation of CaM with Ca^{2+} was performed in $CaCl_2$ solution. All the experiments were carried out in 2×10^{-3} mol/L $Fe(CN)_6^{3-/4-}$solution, where 0.1 mol /L NaCl was employed as the supporting electrolyte.

Results and Discussion

The adsorption process of CaM on Au substrate

Figure 1 is the typical Nyquist plots for the adsorption process of CaM on Au substrate and shows that it contains one semicircle in high frequency region and a 45° line in low frequency region. The diameter of the semicircle represents charge transfer resistance (R_{ct})[6]. The bigger diameter corresponds to the larger R_{ct}. From the plots, we could see that the diameter of the semicircle increased with the adsorption time. So the changing R_{ct} could reflect the varying structure of CaM adsorbed on Au electrode, while the constant R_{ct} standed for the stable structure of CaM. That is to say, R_{ct} could describe the whole adsorption process of CaM on Au substrate to some extent.

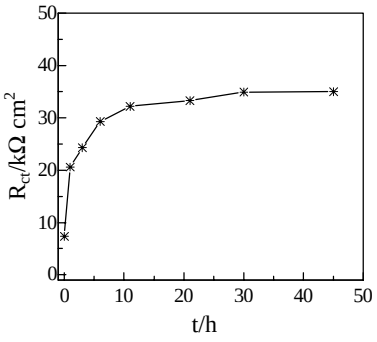
Figure 1 The Nyquist plots for CaM/Au electrode under different adsorption time recorded in 0.1 mol/L NaCl containing 2 mmol/L $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$



(*)0h (o)1h (Δ) 6h (×)21 h (+)30h (□)45h

Figure 2 illustrate the relationship between R_{ct} and adsorption time. From the plot, we can see that R_{ct} increases sharply with adsorption time within 12 h and then it becomes almost constant. One short quick step, which was accomplished within 12 h, and one longer slow step comprise the whole adsorption process. The familiar behavior was observed for alkanethiol[6]. But for alkanethiol, the first quick step could be completed in a few seconds, moreover, its whole process could be finished in several hours[6]. The different adsorption phenomenon between CaM and alkanethiol has just reflected their distinguished molecular structure. Comparing with alkanethiol, CaM structure is very complex. So CaM has to adjust itself to accomplish its adsorption process. The adsorption behavior exhibited by CaM could reflect the common adsorption characterization of protein, which is meaningful to express the protein′s adsorption behavior.

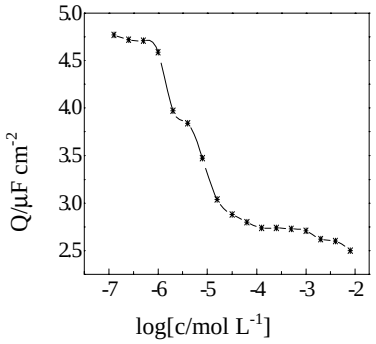
Figure 2 The relationship between adsorption time and the charge transfer resistance (R_{ct})



The complexation of CaM with Ca^{2+}

From **Figure 3**, where C_{app} was obtained from EIS, we could obtain the following information. In the concentration range of Ca^{2+} between 1.25×10^{-7}mol/L and 10^{-2} mol/L, the complexation process could be divided into three steps, two stable stages and one varying region. When the concentration of Ca^{2+} was between 1.25×10^{-7}mol/L and 9.80×10^{-7}mol/L, one stable platform was observed, suggesting that no combining of CaM with Ca^{2+} occurring owing to the lower concentration of Ca^{2+}. C_{app} dropped sharply in the concentration range of Ca^{2+} from 9.80×10^{-7}mol/L to 3.19×10^{-5}mol/L, which accorded with former report very well[6]. When the concentration of Ca^{2+} exceeded 3.19×10^{-5}mol/L, another platform appeared once again, implying that the complexing process of CaM with Ca^{2+} could not be observed any more. In other words, CaM could only complex with certain concentration of Ca^{2+}, which was consistent with the complexing behavior of CaM with Ca^{2+} in the real biological system[7]. All other detailed investigations are being carried out in our laboratory.

Figure 3 The relationship between capacitance and the concentration of Ca^{2+}



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