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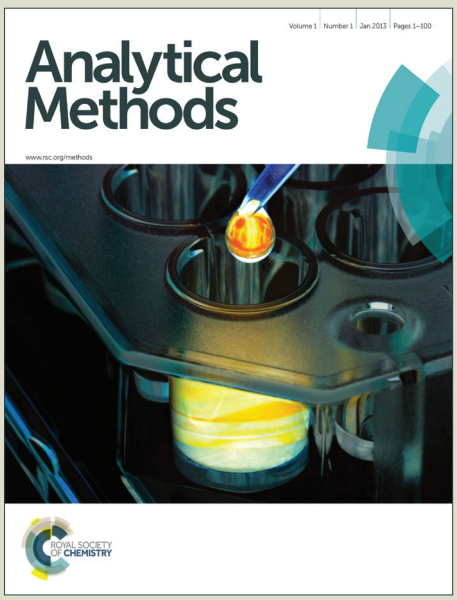

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ARTICLE

Sensitive detection of copper (II) ion based on conformational change of peptide by surface plasmon resonance spectroscopy

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Abstract: Due to the significant role of copper (II) ion in many biochemical and physiological processes, sensitive detection of copper (II) ion has attracted great attention. A novel quantitative copper (II) ion sensor is developed based on the conformational change of Cu^{2+} binding peptides using surface plasmon resonance spectroscopy (SPR). The specific interaction between carboxyl groups of the peptide and Cu^{2+} induces a conformational change from α-helix to β-sheet with decreasing hydrophilicity. The formation of Cu^{2+}-peptides aggregates on the chip surface leads to a refractive index change, which results in a sensitive SPR signal change. Thus, Cu^{2+} can be measured selectively and sensitively due to the conformation change of Cu^{2+} specific binding peptide. With this well-designed sensing platform, the detection range of copper (II) ion is found to be 1×10^{-12} M to 1×10^{-6} M with a detection limit of 0.44 pM, which is 3 orders of magnitude lower than previous reports. The copper (II) ion sensor designed in this study is proposed for application in biological and environmental analysis.

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1. Introduction

Cu^{2+} is an important trace element in living organisms. It plays a significant role in many biochemical and physiological processes at certain concentration, such as in the human gene expression and nervous system [1, 2]. It also plays an essential role in other physiological processes, including energy generation, dioxygen transport and activation, and signal transduction [3-5]. However, at elevated concentrations, Cu^{2+} can react with molecular oxygen to generate reactive oxygen species (ROS), suggesting their potential damage to proteins, nucleic acids and lipids [6, 7]. In addition, the cellular toxicity of Cu^{2+} is related to some neurodegenerative diseases, including Menkes and Wilson's disease, hereditary aceruloplasminemia, prion disease, family amyotrophic lateral sclerosis and Alzheimer's disease [8-10]. The residual level of Cu^{2+} in drinking water is widely restricted in many countries. For instance, the United States Environmental Protection Agency (EPA) gives a drinking water standard for Cu^{2+} of 1.3 ppm to prevent potential health problems [11]. Hence, developing robust environment-friendly methods to measure trace copper with high sensitivity and selectivity has become increasingly important for biological and environmental analysis.

Up to now, there are many approaches for the monitoring of Cu^{2+}, for example, sensors based on organic fluorophores [12, 13], DNAzyme probes [14], atomic absorption spectroscopy [15], inductively coupled plasma mass spectroscopy [16, 17], absorbance spectrophotometry [18], plasma atomic emission spectrometry [19], colorimetric methods based on coordination chemistry [7, 20] and SPR [21, 22]. However, these traditional methods are laborious and time-consuming. Therefore, it is still essential to develop highly sensitive, selective and direct approaches to monitoring Cu^{2+}.

In recent studies, great efforts were focused on the development of short probes, for instance DNA and peptide for capturing metal ions [23-26]. Peptides, which have a good affinity for metal ions, are particularly attractive for the development of chemical sensors in view of the following merits. Peptides, as the simplest biological recognition elements to capture various targets [27], not only allow for cost-efficient large scale synthesis, but also facile modification to produce peptides with enhanced target recognition ability [28]. Due to these merits, peptides have been used as probes for metal ions detection, such as the measuring of Pb^{2+} [28], Cu^{2+} [3, 29], Cd^{2+}, Ni^{2+}, Co^{2+} [30] and Zn^{2+} [23, 31] based on electrochemical method, Fourier transform infrared spectroscopy (FTIR), colorimetric assay and fluorescence.

Surface plasmon resonance spectroscopy (SPR) is a refractive index-sensitive optical technique showing capability to analyze biomolecular interactions [32-34]. It provides an automatic, real-time, no-invasive platform for measuring the analyte [35-37]. Therefore, SPR has attracted research interest not only in basic research but also in some practical fields, including medical diagnostics, food safety and environmental monitoring [38-40].

Ser-Ile-Arg-lys-Leu-Glu-Tyr-Glu-Ile-Glu-Glu-Leu-Arg-Leu-Arg-Ile-Gly (SIRKLEYEIEELRLRIG) is known as a Cu^{2+} specific binding peptide [7, 26], which takes α-helical conformation with good hydrophilicity. The interaction between carboxyl residuals of the peptide and Cu^{2+} induces a conformational change from α-helix to β-sheet, which results in a decreased hydrophilicity and aggregation of the peptides [26]. The Cu^{2+}-binding peptide would therefore selectively aggregate on the chip surface in the presence of copper (II) ions [7, 26]. The Cu^{2+}-peptides aggregates result in a big refractive index change, and can be observed by SPR sensitively (Fig. 1). In this study, four amino acid residues Ac-Cys-Gly-Gly-Gly (Ac-CGGG) as a spacer is designed at the N-terminal of the Cu^{2+}-binding peptide. Peptides were immobilized onto the gold chip surface through Au-S bond. The spacer is used to avoid perturbation of peptides' conformation when covalently immobilized to the surface of Au chip. This copper (II) ions SPR sensor shows excellent selectivity and sensitivity for Cu^{2+} among the metal cations (Mg^{2+}, Ca^{2+}, Zn^{2+}, Pb^{2+}, Mn^{2+}, Ba^{2+}, Ni^{2+} and Co^{2+}) studied.

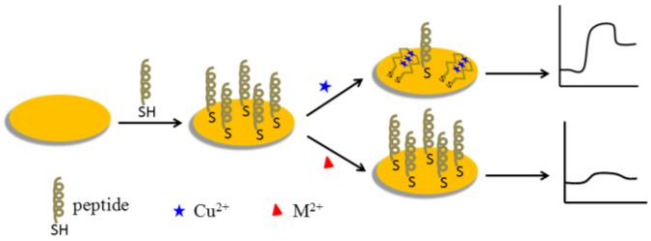

Figure 1 Experimental scheme for the detection of Cu^{2+}.

2. Experimental

2.1 Chemicals and reagents

Tris(2-carboxyethyl) phosphine hydrochloride (TECP), N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) , dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich (St. Louis, Mo, USA). Cu^{2+}-binding peptide with a sequence of Ac-CGGGSIRKLEYEIEELRLRIG-NH_2 was synthesized by Sangon Biotch Co. Ltd. (Shanghai, China). Metal salts of $CuCl_2$, $MgCl_2$, $CaCl_2$, $ZnCl_2$, $PbCl_2$, $MnCl_2$, $BaCl_2$, $NiCl_2$ and $CoCl_2$ were purchased from Sigma-Aldrich (St. Louis, Mo, USA). All chemicals

and solvents were analytical reagents and used without further purification. The ultra-pure water (18.2 MΩ·cm) purified with a Millipore Milli-Q water system (Branstead, USA) was used to make buffer.

2.2 Immobilization of peptide on the gold surface

The Cu^{2+} binding peptide was used without any purification process and dissolved in 10 mM HEPES buffer solution [7] (pH 8.0, containing 10 mM TCEP, 1% DMSO) with a concentration of 1 mg mL^{-1} (~0.41 mM) as stored solution. To immobilize the peptide on the gold surface, a gold disk purchased from Metrohm AG (Hersau Switzerland) or a gold electrode was used as a substrate for immobilization of Cu^{2+} binding peptide. First, the gold chip was soaked in piranha solution (98% H_2SO_4:30% H_2O_2, v/v=3:1) for 5 min to eliminate the adsorbed material and then rinsed with ultra-pure water. Then, the prepared gold chip was fixed onto the SPR instrument. Before peptide immobilization, the bare gold chip was rinsed with 10 mM HEPES buffer in order to balance the gold chip. Then the gold chip was immersed in 1 μM peptide solution (dissolved in 10 mM HEPES buffer, pH 8.0) at 25 ℃ for 2 h. The immobilization process was monitored by SPR spectroscopy. After the immobilization, the gold chip was rinsed with 10 mM HEPES buffer [32].

2.3 Electrochemical measurements

Electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) were carried out on a Metrohm electrochemical analyzer (Metrohm Autolab B.V., the Netherlands) respectively in 5 mM $[Fe(CN)_6]^{3-/4-}$ (containing 0.1 M PBS, pH= 7.0, containing 0.1 M KCl) and 10 mM HEPES (pH 7.0). A three-electrode system consisting of a modified gold electrode (diameter, 3 mm) as the working electrode, a saturated calomel reference electrode (SCE) and a platinum counter electrode was used for all the electrochemical measurements. The whole reaction process on the electrode was measured by EIS and CV. In order to prevent the denaturation of biomolecules on the electrode, after each modification step, the electrode must be used immediately.

2.4 SPR measurements

SPR measurements were performed in an Autolab ESPRIT system (Eco Chemie B.V., the Netherlands) with a 670 nm monochromatic P-polarized light source [41]. The immobilization of the peptide and detection of Cu^{2+} were monitored in real-time at 25℃.

3. Results and discussion

3.1 Characterization of immobilization of peptide and its interaction with copper (II) ion

The EIS and CV are employed to characterize immobilization of peptides on the gold surface and the interaction of Cu^{2+} and peptides. EIS is sensitive to the changes of interfacial properties at electrode surface [42]. Fig. S1 illustrates the EIS of different electrodes in the presence of 5 mM $[Fe(CN)_6]^{3-/4-}$ (containing 0.1 M PBS (pH 7.2) and 0.1 M KCl). The bare gold electrode exhibited a very small semicircle domain (curve a), which means that the charge-transfer process is very fast. After treated with the Cu^{2+} binding peptide, the electron-transfer resistance (Ret) was remarkably increased to 1600 Ω (curve b), which validates the successful assembly of peptides on the gold electrode surface through Au-S bonds. Then, after incubation with 1 μM Cu^{2+}, a slight decrease in impedance arises due to the attraction of copper ions to $[Fe(CN)_6]^{3-/4-}$ (curve c). Fig. 2 shows the CVs for the electrode before and after Cu^{2+} incubation. No redox peaks were observed for the bare gold electrode (curve a) and peptide modified electrode (curve b). However, a pair of well-defined redox peaks were obtained for Cu^{2+}/Cu^{+} with the reduction peak potential at 0.391 mV and a peak potential separation of 0.094 mV after incubation with 1 μM copper chloride (curve c) [42]. These results verify the binding between peptide and copper (II) ion.

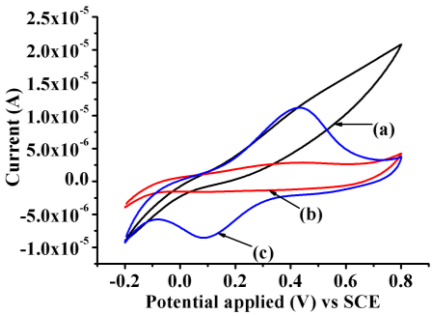

Figure 2 CVs corresponding to (a) bare gold electrode, (b) peptide modified electrode, (c) after incubation with Cu2+. Amplitude: 5 mV; Frequency range: 0.1 Hz to 100 kHz; Scan rate: 100 mV s^{-1}.

SPR spectroscopy was utilized to characterize the immobilization in real time. The peptide can be immobilized on the gold by self-assembly via the covalent bond between Au and the thiol group of cysteine as described elsewhere [32]. The SPR angle increase in millidegrees is used as a response unit to quantify the binding of macromolecules to the sensor surface. A change of 120 millidegrees represents a surface change of approximately 1 ng mm^{-2} as described in previous reports [32]. As shown in Fig. 3, an increase of 470.4 m° in the SPR angle was observed. The surface coverage of peptides on the gold chip is 3.92 ng·mm^{-2} as calculated. These results verify that the peptide was immobilized on the gold surface definitely. To study the interaction the Cu^{2+} specific peptide and Cu^{2+}, 1 μM Cu^{2+} was

injected into the cell. SPR angle increased about 116.5 m°. After rinsing with buffer, no significant SPR signal change was observed, which may due to the high affinity binding between Cu^{2+} and peptides [26]. Though the molecular weight of Cu^{2+} is very low, SPR signal increased dramatically, which may due to the confirmation change of peptides in this binding event. These results confirm the binding event of Cu^{2+} and the immobilized peptide and the peptide modified gold chip is ready to measure Cu^{2+}.

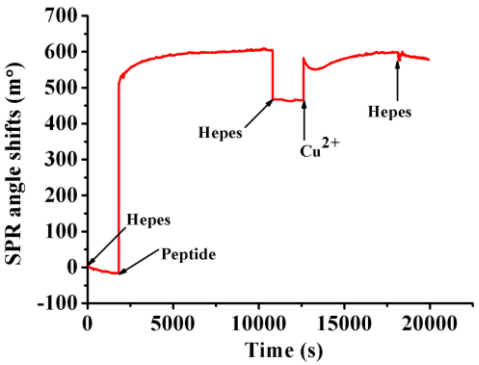

Figure 3 The sensorgram of immobilization processes of peptide on Au-chip and recognition of Cu^{2+}.

3.2 Sensor's selectivity

In order to confirm the selectivity of the peptide modified Au surface, two-channel SPR system was used. The influence of pH on the sensors response was tested using in the pH value range of 4.0–8.0. We found the fabricated sensing system has a higher SPR response near pH 7.0 (data not show), which is consistent with previous report [7]. Thus the optimal value of pH 7.0 was selected in next measurements. Copper (II) ions solution dissolved in 10 mM HEPES [7, 26] with a concentration of 1 μM was injected into the SPR chamber (Experimental group, EG). The control group (CG) was injected with magnesium ions with the same condition and concentration. As shown in Fig. 4A, SPR angle was increased evidently with injection of copper chloride. SPR angle shift for experimental group is 116.5 m°, which indicates that copper (II) ions are captured by the peptide. However, no obvious SPR angle shift was detected for Mg^{2+} as control group. To verify the specific interaction between peptides and Cu^{2+}, same concentration of Cu^{2+} solution was injected onto bare gold chip surface (Fig. 4A). The SPR angle was slightly shifted due the increase of refractive index of Cu^{2+} solution comparing with that of buffer solution. Thus, SPR angle shifts back again after rinsing with buffer, which may due to Cu^{2+} can not adsorb on the bare gold surface. These results verified the peptides can interaction with Cu^{2+} selectively. The big SPR angle change may due to the formation of Cu^{2+}-specific peptide complex, which leads to the refractive index change near the gold film surface [43]. Then, various cations, such as Ca^{2+}, Zn^{2+}, Ba^{2+}, Pb^{2+}, Mn^{2+}, Ni^{2+}, Co^{2+} with the same experimental conditions were injected into the SPR chamber respectively. Fig. 4B shows the histogram of the SPR angle shifts with different ions. These results demonstrated the good selectivity of the peptide to copper (II) ions.

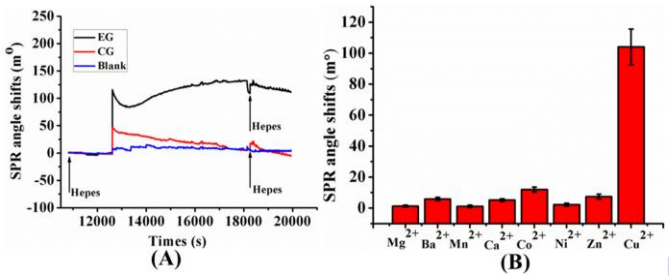

Figure 4 (A) SPR angle shifts according to treatment with Cu^{2+} and Mg^{2+}; (B) SPR angle shifts of various ions compare with copper(II) ions in the same concentration of 1 μM. Error bars represent standard deviations of three independent measurements.

3.3 SPR detection of copper (II) ions

The detection of different concentrations of copper (II) ions was implemented to evaluate the sensing performance. As shown in Fig. 5A, SPR angle increased according to the increasing Cu^{2+} concentrations from 1×10^{-12} M to 1×10^{-6} M. Even at the lowest concentration of Cu^{2+}, SPR angle shifts about 21.29 m°. As shown in Fig. 5B, a linear detection range for the Cu^{2+} is found from 1×10^{-12} M to 1×10^{-6} M (R^2=0.987) with a detection limit of 0.44 pM (LOD=3.3×standard deviation/slope). Comparing with the traditional historical assays, this simple SPR method has a wide detection range and lower detection limit (Table 1). This detection limit is about 2 and 3 orders of magnitude lower than previous sensitive fluorescence [4] and SPR based method [21] respectively. Considering some methods suffering laborious and economic-consuming, the peptide based SPR sensor has significant advantages, such as simple, label-free, real-time, highly sensitive. Therefore, this study offers an accurate and efficient method for copper (II) ions detection.

Table 1 Comparison of analytical properties of the developed SPR sensor with other Cu^{2+} detection methods.

Method	System	Detection range (μM)	Detection limit (μM)	Ref.
Absorption	Dithiothreitol/gold nanoclusters	0-60	0.08	1
	DNA/click chemistry	0.5-10	0.25	3
	Peptide/gold nanoparticles	10-150	1	7
	Starch-stabilized silver nanoparticles	0.1-10	0.5	10
Fluorescence	Branched polyethylenimine/CQDs	0.333-66.6	0.115	2
	DNAzyme/magnetic beads	2×10^{-5}-1.0	1.28×10^{-5}	4
	G-quadruplex/porphyrin protoporphyrin IX	0.008-2.0	0.003	23
	Glutathione protected gold nanoclusters	0.1-6.25	0.086	44
	Coumarin derivative	0.1-1.0	0.015	45
	Single-stranded DNA/ gold nanoparticles	0.625-15	0.29	46
SPR	Cysteamine/peptide	0-1.8	1.6×10^{-3}	21
	Peptide	1×10^{-6}-1.0	0.44×10^{-6}	This study
	Cross-linked chitosan	0-1.5×10^{3}	7.7	48

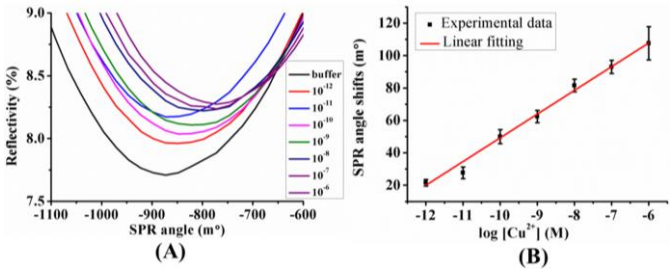

Figure 5 (A) SPR angle shifts according to the incubation with different concentration of Cu^{2+}; (B) Calibration curve for the detection of Cu^{2+} (1×10^{-12} ~1×10^{-6} M). Error bars represent standard deviations of three independent measurements.

4. Conclusions

In summary, a convenient and label-free approach for direct detection of copper (II) ions has been developed, which is based on the conformational change of Cu^{2+}-specific peptide. Compared with previous methods based on fluorescence spectroscopy and colorimetric methods, this simple approach shows excellent selectivity and sensitivity towards copper (II) ions. The detectable range is 1×10^{-12} M~1×10^{-6} M with a detection limit of 0.44 pM. The present approach not only could be used in monitoring Cu^{2+} but also could be extended to other heavy metal ions by employing differential binding peptide. It would be applied in monitoring drinking water and environmental contaminant.

Acknowledgements

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Sensitive detection of copper (II) ion based on conformational change of peptide by surface plasmon resonance spectroscopy

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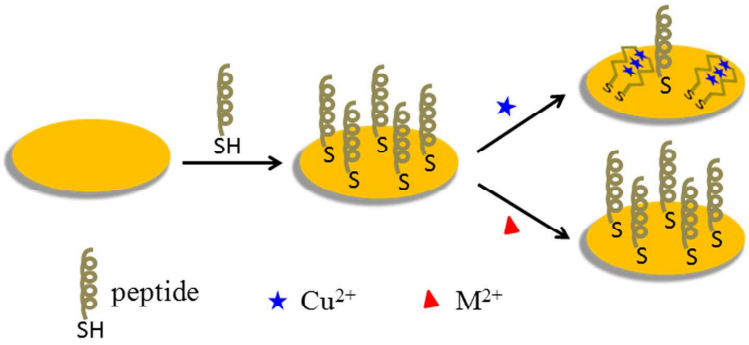

Schematic diagram of sensor chip configuration

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