Formation of Some Cysteine-Containing Peptide Monolayers on Au Electrodes and Their Applications for Metal Ion Sensing and Electrocatalytic Reactions

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The use of biological molecules that already exist in nature offers advantages over using artificially made molecules in developing biosensors or electrocatalysts. Since those molecules have already been best adopted by nature to carry out specific functions, a lot of practical benefit could be acquired by utilizing or mimicking them rather than synthesizing complex molecules. In this regard, amino acids and peptides are favorite systems in metal ion sensing in a sense that they can act as very effective and often specific ligands for a variety of metal ions.[1-6] While amine and carboxyl groups of amino acids participate in complexation, an amide nitrogen atom is also involved in complexation in the case of peptides. This often forms a chelate and a peptide-metal ion binding becomes significantly stronger as a result. Detailed accounts have been treated by Sigel and Martin[7] and other authors.[8]

Despite the fact that amino acids and peptides readily form complexes with metal ions, electrochemical study of those complexes is scarce. It was not until recently when a first report by Yang *et al*.[9] appeared for the electrochemical metal ion detection. They synthesized a tripeptide system, Gly-Gly-His, on top of an alkanethiol self-assembled Au electrode to detect copper ions. Since then, many papers regarding various electrochemical aspects of a peptide-metal binding have been published.[10-12] Here we present our preliminary results of metal ion sensing and electrocatalytic reactions using cysteine-containing simple peptide monolayers constructed on a Au surface. Cysteine was chemisorbed on the Au surface *via* Au-S bonding and other amino acids were formed by the usual peptide synthesis method. This way two types of peptide monolayers were prepared: With a Au/GSH-His system (GSH: glutathione, γ-L-glutamyl-L-cysteinyl-glycine), copper ions of nano molar concentration were detected by accumulating Cu^{2+} on the peptide monolayer. With a Au/Cys-Cys-Cys/M(M = Cu^{2+}, Fe^{2+}) system, electrocatalytic oxidation of ascorbic acid (AA) and reduction of hydrogen peroxide were performed.

Figure 1 shows cyclic voltammograms of a Au/GSH-His/Cu system after immersing a Au/GSH-His electrode in a Cu^{2+}-containing solution and transferring into the Cu^{2+}-free electrolyte. Fairly stable voltammograms were obtained. Without copper ion, no faradaic current was observed. Redox peaks at +0.33 and +0.010 V are assigned to the 1-e^- redox reactions of a Cu^{2+}/Cu^+ couple as indicated in other studies.[13,14] With only GSH layer on Au, current slightly decreased upon multi potential cycling. This means copper ions form a more stable complex with histidine in which imidazole moiety of histidine is involved in complexation. Full copper ion accumulation was complete after *ca.* 10 min. Treating the Au/GSH-His/Cu electrode with EDTA solution, copper ions were completely removed from the modified

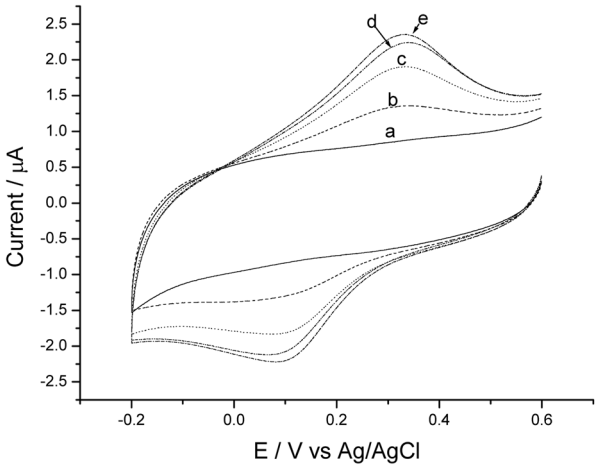

Figure 1. CVs of a Au/GSH-His/Cu system as a function of immersion time: 0 (a), 1 (b), 4 (c), 8 (d), and 10 min (e). The electrolyte was pH 7.0 acetate buffer. [Cu^{2+}] = 0.1 mM. Scan rate = 100 mV s^{-1}.

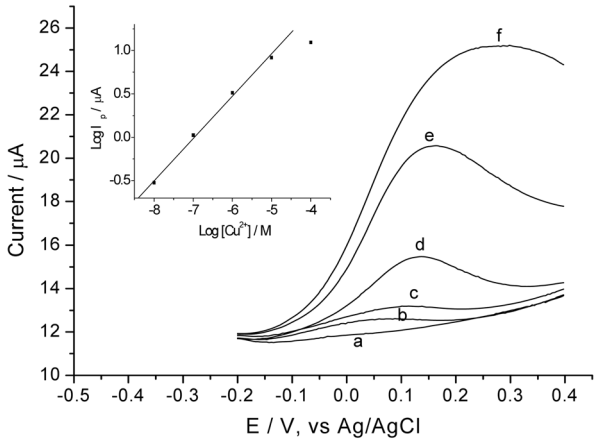

Figure 2. Copper ion accumulation tested with Osteryoung square wave voltammetry for Cu^{2+} concentrations of 10^{-9} (a), 10^{-8} (b), 10^{-7} (c), 10^{-6} (d), 10^{-5} (e), and 10^{-4} M (f). OSWV was done by applying square wave of 4 mV step height and 60 mV pulse height with 60 Hz frequency. Inset: Plot of Log i_p *vs* Log [Cu^{2+}]. Sampling time = 8.3 ms.

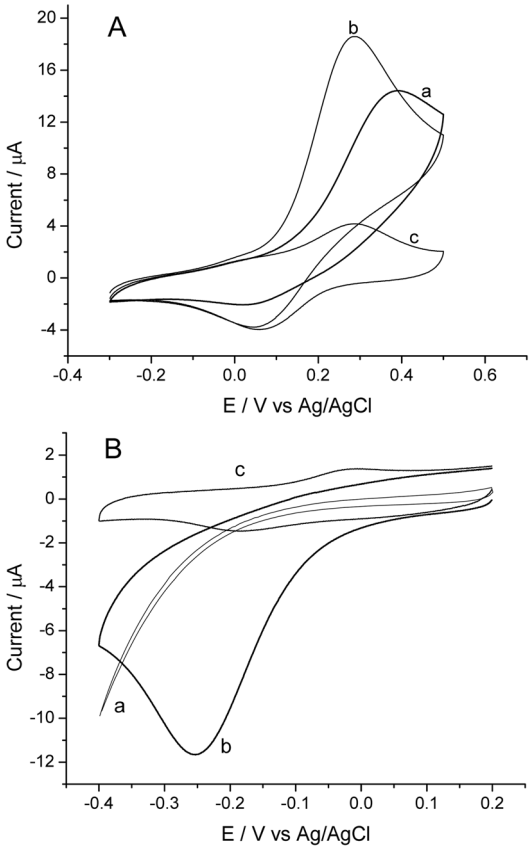

Figure 3. (Panel A) Cyclic voltammograms of AA oxidation on a bare Au (curve a) and on a Au/Cys-Cys-Cys/Cu electrode (curve b). Curve c represents a voltammogram of a Au/Cys-Cys-Cys/Cu electrode. The electrolyte was buffered at pH 7.0. [AA] = 0.91 mM. Scan rate = 50 mV s^{-1}. (Panel B) Cyclic voltammograms of H_2O_2 reduction on a bare Au (curve a) and on a Au/Cys-Cys-Cys/Fe electrode (curve b). Curve c represents a voltammogram of a Au/Cys-Cys-Cys/Fe electrode. [H_2O_2] = 3 mM.

surface since the formation constant of Cu^{2+}-EDTA is much larger than that of Cu^{2+}-peptide. Almost identical voltammetric features were obtained when the electrode was immersed back in a Cu^{2+}-containing solution, indicating that the peptide layer remained intact (data not shown). Even very low concentrations of copper ions can be determined by accumulating them onto the electrode. Figure 2 shows Osteryoung square wave voltammetry of various Cu^{2+} concentrations. Copper ions down to 10^{-8} M (0.64 ppb) could be detected with at least 3 orders of magnitude linearity. Deviation at higher concentrations indicates that the copper binding sites of peptides are saturated with Cu^{2+} ions.

Figure 3 shows Au/Cys-Cys-Cys/M (M = Cu^{2+}, Fe^{2+}) electrodes can be used as an electrocatalyst for the oxidation of ascorbic acid[15] (Panel A) and reduction of hydrogen peroxide[16] (Panel B). Metal ion complexation was done by immersing the Au/Cys-Cys/Cys electrodes into the Cu^{2+} and Fe^{2+}-containing solutions. A Au/Cys-Cys-Cys/Cu^{2+} electrode was used for AA oxidation. On a bare Au electrode AA is irreversibly oxidized at +0.39 V (curve a, Fig. 3A). The modified electrode showed a redox couple corresponding to a Cu^{2+}/Cu^{+} redox reactions at +0.06 and +0.28 V (curve c). Upon addition of AA, AA undergoes electrocatalytic oxidation catalyzed by Cu^{2+} species formed by the oxidation of Cu^{+} according to the following reaction (curve b).

$$\text{Au/Cys-Cys-Cys/Cu}^{+} \rightarrow \text{Au/Cys-Cys-Cys/Cu}^{2+} + e^{-}$$
$$2\text{Au/Cys-Cys-Cys/Cu}^{2+} + \text{HA}^{-} \rightarrow 2\text{Au/Cys-Cys-Cys/Cu}^{+} + \text{D} + \text{H}^{+}$$

where HA^{-} and D represent AA and an oxidized form of AA, respectively. Catalytic current begins to appear as Cu^{+} is oxidized to Cu^{2+}. Peak potential was shifted by 100 mV from 0.39 to 0.29 V, indicating Au/Cys-Cys-Cys/Cu is a good electrocatalytic system.

Panel B shows the reduction of hydrogen peroxide by Au/Cys-Cys-Cys/Fe^{2+}. This electrode displays redox peaks at –0.25 and –0.0 V corresponding to the Fe^{3+}/Fe^{2+} couple in the absence of H_2O_2 (curve c). In 3 mM H_2O_2 solution, a large reduction peak appeared at –0.25 V (curve b) according to the following reaction. Catalytic current begins to appear as Fe^{3+} species is reduced to Fe^{2+}. However, no appreciable current flows on a bare Au electrode (curve a).

$$\text{Au/Cys-Cys-Cys/Fe}^{3+} + e^{-} \rightarrow \text{Au/Cys-Cys-Cys/Fe}^{2+}$$
$$2\text{Au/Cys-Cys-Cys/Fe}^{2+} + \text{H}_2\text{O}_2 + 2\text{H}^{+} \rightarrow 2\text{Au/Cys-Cys-Cys/Fe}^{3+} + \text{H}_2\text{O}$$

In this communication, we have shown several examples to convince that peptide-modified electrodes are very useful in metal ion sensing and in electrocatalytic reactions. Taking full advantages of diverse peptide chemistry, peptide-modified electrode systems will find more useful applications in real world.

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