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「アジアへ広がる電気分析化学の真髄」

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# SI1 Fundamentals and Applications of Redox Enzyme-functionalized Electrode Reactions

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Oxidoreductases (redox enzymes) are concerned with widespread biological electron transfers, *i.e.* processes of respiration, fermentation, and photosynthesis to catalyze electron transfer reactions across the tree of life and to facilitate biologically driven fluxes of several elements such as hydrogen, carbon, nitrogen, oxygen, and sulfur on Earth. The kinds of the redox enzymes are approximately one-quarter of all known enzymes based on enzyme nomenclature.

Redox enzymes use a palette of redox components called coenzymes, prosthetic groups, or cofactors:  $\beta$ -nicotinic amide dinucleotides (phosphate) (NAD(P)<sup>+</sup>), flavins, quinones, hemes, iron-sulfur clusters, copper, molybdenum, nickel, etc. NAD<sup>+</sup>- and NADP<sup>+</sup>-dependent enzymes catalyze hydride ion transfers in main streams of catabolism and anabolism, respectively. Core redox enzymes are ancient and highly diverse in amino acid sequence, and usually require specific transition metals in their active site to catalyze (multi-step) single-electron transfers. On the other hand, flavoproteins and quinoproteins can catalyze both two-step single-electron transfer and hydride ion transfer and link the electron transfer between several organic redox substances and inorganic redox-centers in metal-containing redox enzymes.

Over the last four decades, redox enzymes have received much attention for use in the coupling of the enzyme reactions in electrochemical reactions. The coupled reaction is known as bioelectrocatalysis. It has become an important technology that can be applied to a wide range of bioelectrochemical devices including biosensors, biofuel cells, and bioreactors.

In this lecture, electrochemistry of several quinone cofactors is briefed. An overview of the basic concepts of steady-state catalytic waves of mediated- and direct electron transfer (DET)-type bioelectrocatalysis is also presented. Several equations that can be used for the analysis of steady-state waves are introduced. The analysis may provide important thermodynamic and kinetic parameters that can be used not only for performance evaluation of the devices but also for fundamental research on the enzymes. Important progress made on how to tune electrode surfaces and enzymes for DET-type reactions are presented. Applications to bioelectrochemical devices are also summarized with emphasis on the achievements recorded in my research group.

# S1 Spectroelectrochemical Study on Reduction Processes of Noble Metal and Actinide Ions

Akihiro Uehara (National Institute of Radiological Sciences, National Institutes for Quantum and Radiological Science and Technology, Chiba, Japan)

A fundamental analysis of a metal reduction process is of important for the development on metal plating, electric refining, and nanoparticle synthesis. Electrochemical and spectroelectrochemical methods have been applied for the mechanistic analysis of the noble metal nanoparticles formation as well as the actinide reduction. Ionic species and electron transfer reactions at the interface between aqueous and organic solutions were measured by charge transfer voltammetry at liquid|liquid interface. Chemical status and redox characteristics of actinide ions in various solutions such as water, organic solutions, molten salts, highly concentrated electrolytes were studied by electrochemical methods combined with in-situ X-ray absorption spectroscopy.

**(1) Brust-Schiffrin nanoparticle synthesis** The mechanism of the Brust-Schiffrin gold nanoparticle synthesis has been investigated through the use of ion transfer voltammetry at the water|organic solutions interface, X-ray absorption fine structure in the reaction between  $[\text{AuCl}_4]^-$  and thiol in homogeneous organic solution. The species  $[\text{AuBr}_4]^-$  is shown to be a preferable precursor in the method as it is more resistant to the formation of Au(I) thiolate species than  $[\text{AuCl}_4]^-$ . This new mechanistic understanding should enable nanoparticle synthesis with a higher yield and reduce particle size poly dispersity.

**(2) Metal deposition at the liquid|liquid interface** Electron transfer was observed voltammetrically at the interface between water containing both Au precursors and organic solution containing triphenylamine as reducing agent. Au particles, formed by constant potential electrolysis at the water|organic solutions interface, were examined by transmission electron microscopy. It was shown that deposit size was controlled via the applied potential and time, with specific conditions to form particles of less than 10 nm identified. Here, aqueous chloride ion concentrations and pH influence the interfacial reaction through the ligand exchange equilibria between  $[\text{AuCl}_4]^-$ ,  $[\text{AuCl}_3(\text{OH})]^-$  and  $[\text{AuCl}_2(\text{OH})_2]^-$ .

**(3) Spectroelectrochemistry of uranium reduction** The reduction mechanism of uranium U(VI) was investigated by in-situ X-ray absorption spectroscopy during controlled constant current electrolysis. Formal potential for the reduction of  $\text{UO}_2^{2+}/\text{U}^{4+}$  was determined based on equilibrium potential and radial distribution function of uranium.

## S2 **Bioelectrochemical studies on oxidoreductases and natural polyamino compounds**

Kohei Uematsu (Department of Bioscience and Biotechnology, Fukui Prefectural University, Fukui, Japan)

It has been well known that electroanalytical methods are quite useful tools for analysis of biologically-relevant materials, such as quantification and evaluation of the redox potential, and elucidation of the biological functions and properties of the materials. As might be expected, some of the analytical works can only be performed by the electrochemical methods. I have conducted studies for revealing advantages of the electrochemical method, and studies for elucidating the function and property of biological materials using electrochemical methods, specifically (i) electrochemical method for analysis of enzymatic inhibition reaction, (ii) effect of polyamino compounds on enzymatic reactions of glucose oxidase, and (iii) ion transfer voltammetry of polyamino compounds at liquid | liquid interface. In this presentation, I will introduce some of interesting findings in the study of (ii).

### **Effect of polyamino compounds on enzymatic reactions of glucose oxidase**

In the enzymatic reaction, ionic charges of substrates sometimes have a big influence on the enzymatic reaction rates. One of such reactions is the enzymatic reaction of glucose oxidase (GOx). In the enzymatic reaction, the general oxidant is oxygen. However, GOx can also react with various inorganic or organic oxidants, and the reaction rate tends to become slow as the oxidant is more negatively charged. The tendency of the reaction rate is interpretable by the electrostatic interaction between negatively charged GOx and oxidants. If the electrostatic interaction plays a critical role in the enzymatic reaction rate, a modifier of the electrostatic interaction may also be important for determining the reaction rate.

We investigated the effect of natural polyamino compounds,  $\epsilon$ -poly-L-lysine ( $\epsilon$ PL) and its related compounds, on the GOx reaction. These compounds exist as polycation under acidic and neutral pH conditions, and are expected to interact with the negatively charged GOx. Interestingly, the effect of  $\epsilon$ PL on the GOx reaction dramatically changed depending on the charge of the oxidants. The GOx reaction with the negatively charged oxidant was significantly promoted by  $\epsilon$ PL, while the reaction with positively charged one was fairly suppressed by  $\epsilon$ PL. This result suggests that the effect of  $\epsilon$ PL is determined by the electrostatic interaction among GOx,  $\epsilon$ PL and oxidant. The promotion effect of polyamino compound became extremely large under a certain condition. Thus, the promotion effect is applicable to the improvement of the glucose biosensor and to the development of a sensitive analytical method for the polyamino compounds.

# S3 Determination of Bioactive Compounds by Highly Sensitive Electrochemical Detection in Liquid Chromatography

Akira Kotani (School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan)

Various electrochemical detections (ECDs) for determining bioactive compounds were developed to hyphenate with liquid chromatography (LC-ECD) systems, and these LC-ECD systems were shown to have enough sensitivity and precision to apply for the analyses of biological and herbal medicine samples.

## 1. LC-ECD for determining femtogram levels of flavonoids

An LC-ECD is an attractive method for determining bioactive redox compounds, because ECD is highly sensitive and selective for the detection of redox compounds. And, the miniaturization of the entire system using capillary column (i.d., 0.2 mm) was especially contributed to establish highly sensitive LC-ECD, resulting in electrochemical determinations of catechins and baicalein at femtogram levels.<sup>1)</sup> This LC-ECD was applied to examine the time courses of catechin concentrations in human plasma after green tea administration.

## 2. Multi-channel LC-ECD for analysis of herbal medicines

In order to simultaneously analyze the compounds that possess different hydrophobic properties with good separation, high sensitivity and efficiency, an LC with multi-channel isocratic elution ECD (LC-*n*ECD) was proposed using a new design of channel connections and the technique of alternate rotations of switching valves. Phenylethanoid glycosides in *Magnolia officinalis* roots and caffeoylquinic acids and flavonoids in *Chrysanthemum morifolium* flowers were determined by an LC-2ECD<sup>2)</sup> and an LC-3ECD,<sup>3)</sup> respectively, and quality assessment of herbal medicines were performed by these LC-*n*ECD systems.

## 3. LC-ECD for determining electro-inactive acids based on the reduction of quinone

Voltammetric acid sensing based on the reduction of 3,5-di-*tert*-butyl-1,2-benzoquinone (DBBQ) was applied to determine electro-inactive acid compounds, such as valproic acid (VPA) and polyunsaturated fatty acids (PUFAs). The present ECD was hyphenated with an LC system, and the LC-ECD system was provided as a quantitative analytical method for pharmacokinetic studies of VPA and PUFAs in plasma.<sup>4,5)</sup> Moreover, a novel electroanalytical method for determining electro-inactive amino acids was proposed by the present method coupled with a concept of back neutralization titration.<sup>6)</sup>

In conclusion, these LC-ECD systems with sensitivity produced a practically useful method for determining bioactive compounds in biological and herbal medicine samples.

**Reference:** <sup>1)</sup> *J. Pharm. Biomed. Anal.*, 2008. <sup>2)</sup> *J. Pharm. Biomed. Anal.*, 2018. <sup>3)</sup> *Anal. Sci.*, 2015. <sup>4)</sup> *J. Pharm. Biomed. Anal.*, 2014. <sup>5)</sup> *Anal. Sci.*, 2016. <sup>6)</sup> *Electroanalysis*, 2018.