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Functional Electrodes for Protein Electrochemistry and Their Applications

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Electron transfer reaction is one of the key reactions for generating a biological function: Preparation of a suitable electrode/solution interface, at which rapid electron transfer reaction of metalloprotein takes place, is important not only for studying biological functions of metalloproteins using conventional electrochemical techniques but also for applying biological functions to design various bioelectrochemical systems including bio-fuel cells [1, 2]. When a suitable electrode for probing metalloproteins is obtained, various biological functions related to electron-transfer reactions can be measured electrochemically [3-5]. Although still many proteins remain unsuccessful to obtain rapid electron transfer at an electrode, various functional electrodes for protein electrochemistry have been developing. Also, in recent years, functional electrodes using single crystal electrode surfaces has been examined extensively, and surface functions of the modified electrodes became more and more clear at the molecular level [6-13].

In the present paper, recent developments on useful functional electrodes for metalloprotein electrochemistry and bioelectrochemical systems are discussed.

Functional Modified Electrodes for Cytochrome c Electrochemistry

Cytochrome c is one of the most extensively studied proteins so far used in metalloprotein electrochemistry. Indium oxide (In2O3) and so-called electron transfer promoter (such as bis(4-pyridyl)disulfide, 4,4'-PySSPy and 4-mercaptopyridine, 4-PySH) modified electrodes are typical examples at which a well-defined voltammogram of cytochrome c is clearly seen [1,2]. More recently, using the atomically flat gold single crystal surfaces, surface structures of various modified electrodes have been examined at the molecular level [6]. For example, the STM image of the 4-PySH modified Au(111) electrode showed the rectangular unit cell of a p(5× 3R-30) structure with an interaction of two neighboring 4-PySH [7-10]. On the other hand, the 2-PySH modified Au(111) electrode showed a p (4 x 7R-40.9) structure with no dimer formation, suggesting that 2-PySH adsorbed at both thiolate S and pyridine N atoms [11-13]. On a 2-PySH modified electrode, poor electrochemical response of cytochrome c was seen as was on a thiophenol modified electrode. The 2-PySH modified surface has no pyridine nitrogen at the solution side, while the 4-PySH modified surface has pyridine nitrogen faced to the solution, through which cytochrome c in solution interacts with the electrode. Similar disulfide formation for 4-PySH (p (2R-45 ×5R-53.1)) and adsorption at both thiolate S and pyridine N atoms for 2-PySH ((2 x 3 2) R-45) were also observed, respectively, on 4-PySH and 2-PySH modified Au(100) electrodes, where 2-PySH modified Au(100)electrode showed no electrochemical response. These results suggest that for the rapid electrochemical response of cytochrome c, the pyridine nitrogen faced to solution is necessary. This consideration for the suitable structure of the electrode surface, was confirmed by using 3-mercaptopyridine

modified Au(111) and Au(100) electrodes, where excellent voltammetric responses for cytochrome c was seen [2]. The STM images of the surface of 3-PySH modified Au(111) electrodes suggested a (6 x 3R-30) structure. The observed surface structures obtained by modifying with mercaptopyridines were the same as those obtained by modifying with corresponding disulfides [7-13].

Probing Biological Functions of Myoglobin (Mb)

Mb is an interesting molecule, because its redox center (an iron complex of protoporphyrin-IX, protoheme-IX) is not covalently bonded to the globin moiety and is easily replaced with an artificial molecule for the redox center. Also, structure of Mb is well known on the basis of x-ray crystallographic studies. Recently, binding of exogenous ligands and properties of semi-artificially reconstituted Mbs have been studied electrochemically [14, 15]. Electrochemical properties of various mutated Mbs were also measured at an In2O3 electrode. For example, electrochemistry of Mb reconstituted with symmetric protoheme-III and protoheme-XIII [16], instead of asymmetric protoheme (protoheme-IX) for native Mb, has been examined. The electrochemical behavior and biological functions obtained showed that the difference in symmetry for the heme structure gives no significant change in Mb function.

Use of Ferredoxin (Fd) Electrochemistry for Bioelectrocatalytic Reactions

Since Fd is an electron-transfer protein for photosynthetic process and reduced Fd acts as electron-donating mediator for various enzyme reactions, electrochemically activated Fd is applicable to develop various bioelectrocatalytic reactions as in nature. The modified In2O3 electrode is optically transparent and easy to construct a cell to monitor spectroscopically the reactions during electrolysis [5]. For example, in the presence of Fd-$NADP^+$-reductase (FNR, E.C.=1.18.1.2), NADPH formation was monitored as an increase in absorbance at 340 nm with the current efficiency of near 100%. Because chlorella Fd is thermostable up to 70 C, chlorella Fd is easily handled for such reactions. By further conjugation with other enzyme reactions, various biocatalytic reactions are also designed. In addition, Fd was successfully immobilized on the electrode surface by casting poly-lysine and then Fd into the polymer matrix and drying. Similarly both Fd and FNR were immobilized on the electrode surface. This electrode, for example, was applied to analyze $NADP^+$ in solution using observed catalytic current at –0.8 V (vs. Ag/AgCl). NADH formation was also confirmed spectroscopically.

Maize Fd of which particular amino acid residues had been modified by site-directed mutagenesis showed well-defined cyclic voltammograms at a modified electrode for Fd electrochemistry. Electrochemical study showed particular evolutionary conserved amino acid residues have distinguished roles in biological functions. [17]

Construction of Bio-Fuel Cells

Various bio-fuel cells can be prepared by suitable combination of bioelectrochemical reactions. Using direct and indirect electrochemistry of metalloproteins and enzymes bio-fuel cells have been designed. For example, glucose oxidation [18] was combined with oxygen reduction to prepare "sugar-air battery". The stable tetrathiafulvalene (TTF) modified carbon electrode gave oxidation current of glucose around 0 V (vs. Ag/AgCl) with the aid of glucose oxidase (GOD) in a phosphate buffer solution at pH 5-7. This reaction can be combined with oxygen reduction with the aid of bolirubin oxidase (BOD) at a carbon electrode (for example, in the presence of 2,2'-azino-bis(3-ethylbenzo-)thiszoline-6-sulfonic acid (ABTS) or other catalytic electrodes used for air battery (Imax=0.1 mA, Vmax=0.45 V, and P max=12 μW) to give a bio-fuel cell. Although in this case the current, voltage and power obtained were still rather low, promisingly better combinations improves the battery performance.

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