Asymmetric Electrosynthesis of Amino Acid Using an Electrode Modified with Amino Acid Oxidase and Electron Mediator

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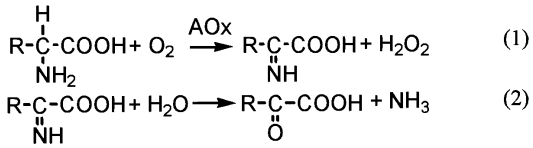
Asymmetric synthesis of amino acid has been successfully achieved by electrochemical reduction of keto acid using an electrode on which amino acid oxidase and electron mediator are immobilized. The enantiomer excess closing to 100% was obtained

Electrochemical utilization of enzymes has been extensively studied from the viewpoint of fabrication of biosensors[1-3] and electroorganic synthesis systems.[4,5] The most successful example among them should be the glucose sensors prepared by using the glucose oxidase (GOx), which have been already commercialized. To accomplish electron communication between the enzyme molecules and an electrode, it is required to use electron mediators. In the case of GOx having a flavin adenine dinucleotide (FAD) as a redox active center, O_2 is the original electron acceptor to induce the enzyme reaction, i.e. oxidation of glucose, but some artificial redox species such as quinone derivatives and metal complexes also were found to make electron exchanges with FAD in the enzyme.[6] Such findings allowed preparation of reagentless glucose sensors by immobilizing GOx together with those redox species as electron mediators on an electrode.[1,2]

The amino acid oxidase (AOx) is another enzyme having FAD as a redox active center. This enzyme catalyzes in-vivo oxidation of amino acid to imino acid in the presence of O_2.[7] The produced imino acid is hydrolyzed, giving keto acid and ammonia in an aqueous solution, as given by the following reactions.



Hydrogen peroxide produced by the reaction (1) is decomposed by the catalase in living organisms. If an appropriate electron mediator is available, it is expected to induce electrochemical oxidation of amino acid as the case of the glucose sensor. Furthermore, since this enzyme reaction is known to proceed under equilibrium conditions in the absence of the catalase,[7,8] it is also expected that the reverse reaction can be electrochemically induced by choosing an electron mediator having the ability to reduce FAD in AOx, as schematically shown in Figure 1. Based on this idea, we have attempted in this study the electrochemical reduction of keto acid to amino acid with the use of an electrode on which both AOx and electron mediator are immobilized. In this communication, it will be shown that the electrolysis using the prepared electrode induces asymmetric production of amino acid with the enantiomer excesses closing to 100%. The electrochemical conversion of keto acid to amino acid has already been attempted by using the amino acid dehydrogenase.[9] In that case,

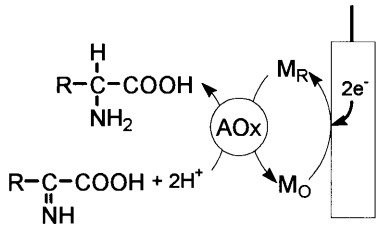


Figure 1. Electrochemical synthesis of amino acid with use of AOx and electron mediator. M_R and M_O denote an electron mediator in its reduced and oxidized states, respectively.

necessity to use the nicotinamide coenzyme (NAD(P)) and another enzyme catalyzing reduction of NAD(P) seems to be undesirable for preparation of an electrode equipped with all required species.

In order to induce the reverse reaction, it is essential to use the electron mediator having a redox potential which is more negative than that of FAD (–0.15 V vs. Ag/AgCl). It is also better for the electron mediator to possess an appropriate functional group which is available for its chemical immobilization. With those in mind we chose in this study 1-aminopropyl-1'-methyl-4,4'-dipyridinium iodide (ADPy), which was prepared by quaternization of 4,4'-dipyridine with methyl iodide and 3-aminopropyl iodide. D-Amino acid oxidase (D-AOx: Type X) from *Procine Kedney* and L-amino acid oxidase (L-AOx: Type IV) from *Croalus adamanteus venom* were commercially available from SIGMA and used as received. A glassy carbon plate (Tokai Carbon) having an exposed area of 1.0 cm^2 was used as an electrode substrate. Its surface was successively polished with 1 and 0.3 μm alumina particles slurry to obtain the mirror-finished surface. The immobilization of AOx and ADPy was conducted by using glutaraldehyde as a cross-linking agent in a similar manner as the case of the immobilization of other enzymes and electron mediators.[2,3] Phosphate buffer (pH 6.5, 0.1 ml) containing 10 nmol ADPy, 21.2 μmol glutaraldehyde, and 1 unit of D-AOx was cast on the electrode surface, followed by drying for ca. 10 h in a glass vessel filled with dry N_2 at room temperature. The prepared electrode is denoted here as D-AOx/ADPy/GC.

Cyclic voltammetry of the D-AOx/ADPy/GC was taken in 0.1 mol dm^{-3} phosphate buffer (pH 6.5). The voltammogram obtained at the potentials ranging between –0.7 V and 0 vs. Ag/AgCl showed a couple of anodic and cathodic current peaks that located at –0.23 and –0.43 V vs. Ag/AgCl, respectively. Since the average of the peak potentials (–0.33 V vs. Ag/AgCl) was well in accordance with the redox potential of ADPy (–0.34 V vs. Ag/AgCl) dissolved in an aqueous solution, the redox waves observed for the D-AOx/ADPy/GC electrode was assignable to the redox reaction of ADPy immobilized on the electrode surface. Integration of the reduction wave of the voltammogram allowed estimation of the amount of ADPy on the electrode to be 9.8 nmol

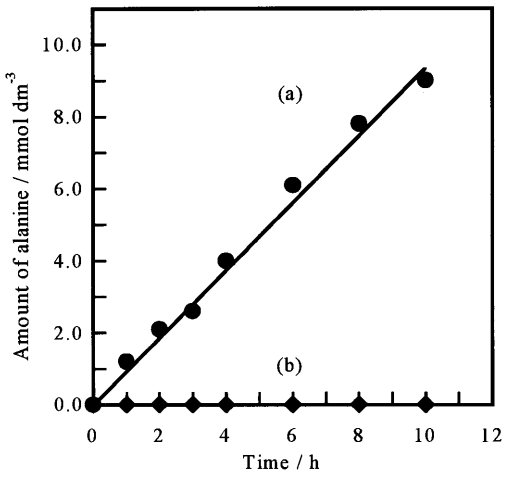


Figure 2. Production of D-alanine (a) and L-alanine (b) by electrochemical reduction at –0.7 V vs. Ag/AgCl of 0.1 mol dm^{-3} phosphate buffer (pH 6.5, 20 ml) containing 30 mmol dm^{-3} pyruvic acid and 30 mmol dm^{-3} NH_4OH with the use of D-AOx/ADPy/GC electrode.

which accorded well with that of ADPy which was mounted on the electrode in the preparation of the electrode. The activity of D-AOx immobilized on the electrode was determined by the chemical oxidation of D-alanine in the presence of O_2 using the prepared D-AOx/ADPy/GC as a catalyst. The obtained activity of 0.55 units was smaller than the original activity of 1.0 unit, suggesting that some inactivation of the enzyme molecules happened by their immobilization.

The electrochemical reduction of pyruvic acid was conducted with the use of the D-AOx/ADPy/GC electrode. The electrolysis experiment was carried out using a two-compartment cell separated by a cation exchange membrane (Nafion 117, Aldrich). The electrolyte solution used was 0.1 mol dm^{-3} phosphate buffer (pH 6.5, 20 ml) containing 30 mmol dm^{-3} pyruvic acid, 30 mmol dm^{-3} NH_4OH. The identification of products and determination of their amounts were made by using a high-performance liquid chromatography with a Sumichiral OA-5000 column. Figure 2 shows the time course of alanine production obtained by the electrolysis at –0.7 V vs. Ag/AgCl. As recognized, the product of D-alanine increased with the electrolysis time, whereas no L-alanine was produced, indicating that the electrochemical reduction of pyruvic acid took place according to the reaction scheme shown in Figure 1. The HPLC analysis gave the enantiomer excess of 100% for the production of D-alanine. The enantiomer excess larger than 99% was also obtained by the polarimetric analysis of the electrolyte solution that was subjected to the electrolysis for 10 h. The current efficiency larger than 97% was obtained through the electrolysis experiment. Production of 8.9 mmol dm^{-3} D-alanine in 20 ml of the electrolyte solution allowed estimation of the turnover number more than 36,000 for the immobilized ADPy. Those results indicated clearly that the D-AOx immobilized on the electrode kept its high selectivity for the asymmetric reaction and it functioned well even for the reverse reaction induced by the electron mediation of the immobilized ADPy.

The electrolysis experiments were conducted under some different conditions. Changes in the polarization potential between –1.2 and –0.4 V vs. Ag/AgCl and changes in the amount of the immobilized ADPy between 5 and 20 nmol did not influence significantly the rate of D-alanine production. However, as shown in

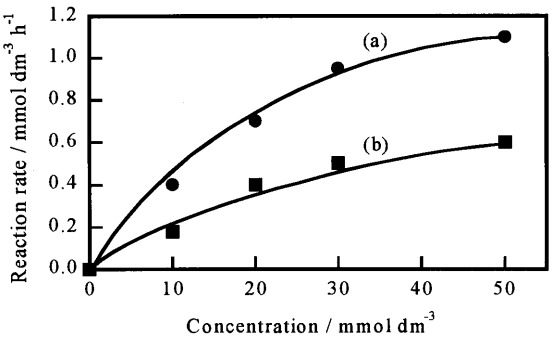


Figure 3. Plots of reaction rate of electrochemical reduction of pyruvic acid to D-alanine using the D-AOx/ADPy/GC electrode as a function of concentration of pyruvic acid and NH_4OH. Amount of AOx immobilized on the electrode used was 1 and 0.5 units for the results (a) and (b), respectively.

Figure 3, the reaction rate was enhanced by increasing the concentration of substrates (i.e. pyruvic acid and NH_4OH) and the amount of AOx immobilized on the electrode. It is then suggested that the reduction of substrates at AOx was the rate-determining step under the condition chosen in this study.

Selective production of amino acid having L-configuration was also successfully achieved by the electrochemical reduction of phenylpyruvic acid using the L-AOx/ADPy/GC electrode. The electrode was prepared by the same method as the case of the D-AOx/ADPy/GC except for dissolution of 1 unit of L-AOx instead of D-AOx in the casting solution. The electrolyte solution used was 0.1 mol dm^{-3} phosphate buffer (20 ml) containing 30 mmol dm^{-3} phenylpyruvic acid and 30 mmol dm^{-3} NH_4OH. The electrolysis at –0.7 V vs. Ag/AgCl for 10 h gave 8.5 mmol dm^{-3} L-phenylalanine and no detectable D-phenylalanine.

The electrochemical production of L-alanine using L-AOx/ADPy/GC and that of D-phenylalanine using D-AOx/ADPy/GC were also attempted. However, significant amount of products was not obtained. The L-AOx (Type IV) and D-AOx (Type X) used in this study are known to possess the substrate selectivity for phenylalanine and alanine, respectively, in the original oxidation reaction. It is then likely that the substrate selectivity of the enzymes also limits kind of the electrochemically induced reverse reaction. If one would like to use this electrolysis system to obtain several kinds of amino acids, enzymes having lower substrate selectivity should be desirable. Experiments focusing on such further exploration are underway.

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