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Electroenzymatic Oxidation of Alcohols on a Poly(acrylic acid)-coated Graphite Felt Electrode Terimmobilizing Ferrocene, Diaphorase and Alcohol Dehydrogenase

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A preparative electroenzymatic oxidation of alcohols was successfully carried out on a poly(acrylic acid)-coated graphite felt electrode ter-immobilizing ferrocene, diaphorase and alcohol dehydrogenase in the domain of the poly(acrylic acid) layer in a 1 mM NADH / phosphate buffer (pH 7.2) with high stereoselectivity (> 96%), high isolated yield (> 90%) and high current efficiency (> 89%).

For a successful preparative bioorganic synthesis using bioelectrochemical methods, a careful design and construction of the reaction system is essential. Steckhan and Frede[1] have reported the electroenzymatic oxidation of p-hydroxytoluene using an electrochemical enzyme membrane reactor in which enzyme (p-cresol-methylhydroxylase) and mediator (water soluble polymer-modified ferrocene derivatives) were confined within an ultrafiltration membrane. Instead of using an electrochemical enzyme membrane reactor, we have succeeded in carrying out the selective electroenzymatic oxidation of NADH to NAD^+ on a modified electrode with high efficiency; the electrode is a thin poly(acrylic acid) (PAA)-coated graphite felt (GF) coimmobilizing two kinds of mediator, ferrocene (Fc) and diaphorase (Dp), in a practical scale.[2] To continue our studies, we have carried out further immobilization of alcohol dehydrogenase into the PAA layer of the above modified electrode and achieved a smooth electrocatalytic oxidation of alcohols which, we think, could be the first report concerning a preparative bioelectrochemical reaction on modified electrode.

Thin PAA film-coated GF electrode immobilizing Fc and Dp in the PAA film was prepared according to the procedure described in the previous report.[2] A piece (5.0 x 2.0 x 0.5 cm^3) of GF (National Electric Carbon Corp., WDF) was immersed in a 0.25% PAA (average MW of 1400000) methanol solution and

Table 1. Some Characteristic Data of Thin PAA Film-coated Graphite Felt Termodified with Fc, Dp and ADH

Ratio of COOH groups of PAA / % [a]					Density of Fc	Oxidation potential V vs. SCE
Cross-linked	Fc-modified	Dp-modified	ADH-modified	Free	μmol / cm^3	
33	46	8	6	7	11.5	0.24

a) The values were calculated by back titration with 0.01 M hydrochloride solution.

dried in air. The PAA film-coated GF (ca. 40 nm thick PAA) was attached with ferrocenylethylamine, crosslinked with hexa-methylenediamine and Dp (Behlinger E.C. 1.8.1.4) successively. The modified GF thus prepared was reacted with 5 ml of 2.72 $U \cdot mg^{-1}$ horse-liver alcohol dehydrogenase (ADH, Behlinger E.C. 1.1.1.1) in phosphate buffer (pH 7.2) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (10 equiv. to ADH) at 4 °C for 48 h and dried in vacuo.[3-5] Some characteristic data of the modified GF are shown in Table 1.

The cyclic voltammogram (CV) (———) of the electrode (1.0 x 1.0 x 0.5 cm^3) in phosphate buffer (pH 7.2) is shown in Fig. 1 together with the CV (- - - - - -) in the presence of 1 mM NADH (Oriental Yeast Co., Ltd.) and the CV (— · —) in the presence of both 1 mM NADH and 50 mM cyclohexanol. An electrocatalytic wave for the oxidation of NADH was observed at the peak of 0.25 V vs. SCE along with a large electrocatalytic wave (three times large peak current comparing with that for NADH) at the peak of 0.3 V., indicating that the electrode is active for oxidation of cyclohexanol.

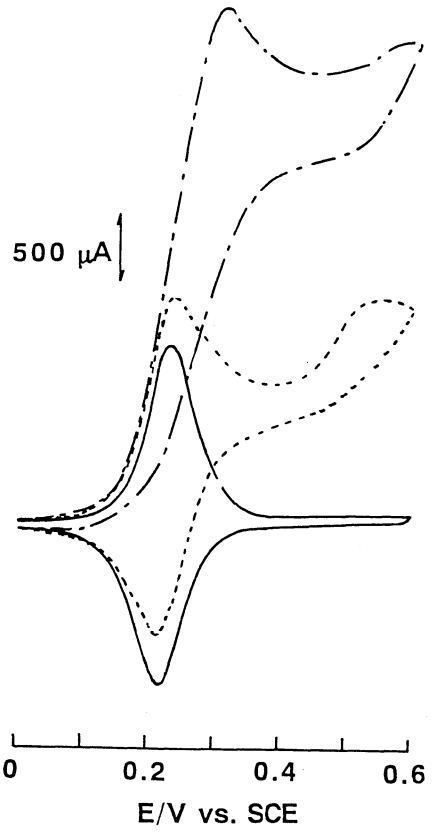


Fig. 1. Cyclic voltammograms on Fc-, Dp-, ADH-termodified graphite felt electrode (1.0 x 1.0 x 0.5 cm^3). ——— : in the absence of both NADH and cyclohexanol, - - - - - : in the presence of 1 mM NADH and absence of cyclohexanol, — · — : in the presence of both 1 mM NADH and 50 mM cyclohexanol in phosphate buffer (pH 7.2). Scan rate: 50 mV/s.

In preparative electrolysis, 50 mM of substrate was electrolyzed in an undivided cell which holds 50 ml phosphate buffer (pH 7.2) containing 1 mM NADH, at the constant potential of 0.30 V vs. SCE under N_2 atmosphere. The electrolysis continued until the current decreased to almost zero and the consumption of the substrate exceeded 99% as confirmed by gas chromatography. After extraction of electrolyte with organic solvents, the reaction products were isolated by distillation and weighed. The purity of the products was analyzed by gas chromatography and / or high performance liquid chromatography, and polarimeter.

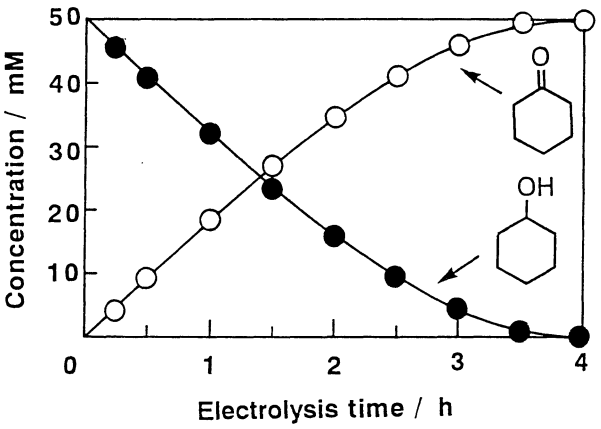
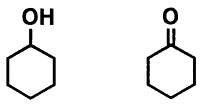
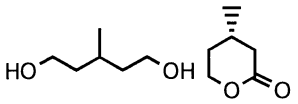
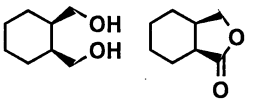
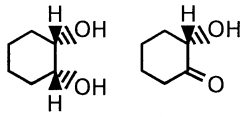


Fig. 2. Macroelectrolysis of cyclohexanol on Fc-, Dp-, ADH-termodified graphite felt electrode (5.0 x 2.0 x 0.5 cm^3) in the presence of 1 mM NADH in phosphate buffer (pH 7.2).

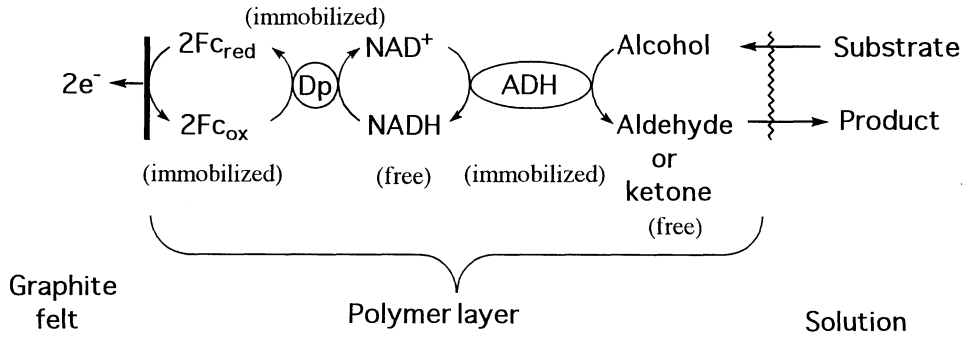
The variations in the concentration of cyclohexanol reduced and the concentration of cyclohexanone formed with electrolysis time, as determined by gas chromatography (column : PEG 20M) are shown in Fig. 2. Fifty millimole of cyclohexanol was completely reacted while almost the equal moles of cyclohexanone was formed after 4 h on the modified electrode (2.0 x 5.0 x 0.5 cm^3) in the presence of 1 mM NADH / phosphate buffer (pH 7.2). The other substrates of 3-methyl-1,5-pentanediol, cis-1,2-cyclohexane-dimethanol and cis-1,2-cyclohexanediol were similarly oxidized. The preparative results of the oxidation of four alcohols are shown in Table 2. All the substrates were oxidized electrocatalytically to form the products as anticipated with high current efficiency (> 89%), high isolated yield (> 90%) and high selectivity (> 96%). The retention of substrate configuration was confirmed in the oxidation of cis-1,2-cyclohexanedimethanol (100%) and cis-1,2-cyclohexanediol (92%). Ninety five precent of stereoselective S(-)-3-methylvalerolactone was obtained from 3-methyl-1,5-pentanediol. These stereoselective results can be understood reasonably, because electro-enzymatic reactions usually proceed in high asymmetry.[6]

Table 2. The Preparative Results of the Electroenzymatic Oxidation of Alcohols on Fc- , Dp- , ADH-Termodified Graphite Felt Electrode by Use of NADH

Substrate	Product	Charge passed[a]/C	Current efficiency/%	Isolated yield/%	Selectivity %	$[\alpha]_D^{20}$	Stereoselectivity %	Turnover number of Fc
		988	96.1	98.4	100[b]	0	0	171
		974	89.4	90.2	96[c]	-27.8°[f]	95[c]	157
		1005	90.8	94.5	100[d]	+48°[g]	100[d]	164
		1018	91.8	96.8	100[e]	-12.2°[h]	92[e]	168

a) Substrates were completely reacted by gas chromatography (100% conversion). b) Extracted with methylene chloride, distilled, and analyzed by GC (column : PEG 20M). c) Extracted with chloroform, distilled, and analyzed by GC (column : CP-cyclodextrin-B-2,3,6-M-19) and HPLC (column : CHIRALCEL OD). d) Extracted with diethyl ether, distilled, and analyzed by GC (column : CP-cyclodextrin-B-2,3,6-M-19) and HPLC (column: CHIRALCEL OD). e) Extracted with methylene chloride, distilled, and analyzed by GC (column : CP-cyclodextrin-B-2,3,6-M-19) and HPLC (column : CHIRALCEL OD). f) Lit.[7] $[\alpha]_D^{20}$ -28.2°. g) Lit.[8] $[\alpha]_D^{25}$ +48.8°. h) Lit.[9] $[\alpha]_D^{20}$ -13.3°.

Thus, the bioelectrocatalytic oxidation of alcohols was successfully attained on Fc-, Dp- and ADH-immobilized GF electrode (Scheme 1). Success in the smooth electron transfer reaction between electrode and alcohol through Fc, Dp, coenzyme of NADH and ADH is probably due to the flexible Fc moieties attached to the domain of the PAA layer and the movable NADH molecules in that of the PAA layer. If flexible NADH moieties are immobilized with the carboxylic acid groups of the PAA layer, a complete bioelectrocatalytic reaction can be attained on the modified electrode. This kind of noble reactions are in progress.



Scheme 1. Mechanism of electroenzymatic oxidation of alcohols on Fc-Dp-ADH-terimmobilized graphite felt electrode.

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