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# Enzyme electrode based on gold-plated polyester cloth

## 2. $NAD^+$ and NADH regeneration by diaphorase-immobilized electrode

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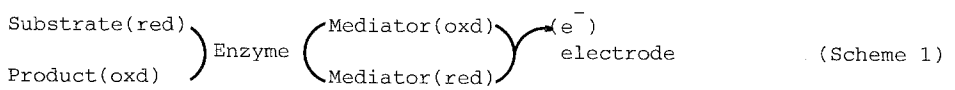
Summary

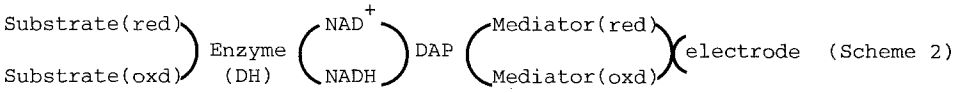
Diaphorase is immobilized on electrodes derived from metal-plated polyester cloth by entrapment method and coupled with an electron mediator. Cyclic voltammogram depended on the existence of the substrate NADH (for oxidation) or $NAD^+$ (for reduction) and the flavo-enzyme was shown to exchange electron(s) with the cloth electrode by mediation of the ferrocene derivative (for oxidation cycle) or the viologen derivative (for reduction cycle), even in the immobilized state. Coupling of the NADH or $NAD^+$ regenerating system with alcohol dehydrogenase was examined.

One of the most important problems for obtaining more effective system to utilize specific and selective catalysis of enzymes in various practical purposes is how to communicate between the biology-origin materials and the energy supplying artificial systems. It is now getting understood that by immobilizing enzymes (directly) on proper electrode with some electron mediators the electron transfer on the electrode surface is controlled by the given electric potential through which the reaction of oxido-reductase can be controlled.(1) Mediators are usually required since the direct electron exchange between the electrode and the enzyme is difficult (Scheme 1). We have shown that in a glucose oxidase (GOD)-immobilized electrodes derived from gold-plated polyester cloth (Au-plat) the flavo-enzyme donated electron(s) to the electrode with mediation of the ferrocene derivative.(2,3)

Another important problem in enzyme electrode, the materials of the electrode, was also challenged there. Most usually used platinum or gold electrode is expensive and inflexible. Carbon fibers are better in these senses but they are still inconvenient to treat (brittle). In contrast the electrodes derived from polyester cloth is advantageous in many points such as flexibility, wide-surface area, processability after enzyme immobilization and cheapness. In the present study another useful flavo-enzyme, diaphorase (DAP) is immobilized. DAP catalyzes NADH oxidation/$NAD^+$ reduction with wide specificity with respect to the electron acceptor/donor and hence several attempts have been made to couple this enzyme with other NADH- or $NAD^+$- requiring oxido-reductase reaction to produce various useful chemical products.(4) The present study is to draw a basis for coupling the cofactor regeneration by electrode system (Scheme 2).




(Scheme 1)




(Scheme 2)

Experimental

Gold-plated polyester cloth (Au-plat) was prepared by chemical plating (Atomex, Nippon Engelhard) of Ni-plated cloth (Seiren Ni-plat). As for the reduction of $NAD^+$ the hydrogen overvoltage required to use carbon-coated electrode, which was prepared by coating the Ni-plated cloth with melamine and acrylate resin containing large amount of carbon particles (<1uM) and by partially scraping off the resin.

Ferrocene carboxylic acid (FcCOOH) was used as a mediator for oxidation reaction and methylviologen (MeV) was for the reduction reaction. Diaphorase (lipoamide oxidoreductase from pig heart, E.C.1.6.4.3; DAP) was purchased from Boehringer Mannheim GmbH ( Lot.11021228-33). Horse liver alcohol dehydrogenase (E.C.1.1.1.1; ADH) was from Sigma Chem.Comp.(Lot.58F-8010). Other reagents were commercially available.

Enzyme was immobilized on the electrode by using urethane-acrylate prepolymers (donated by Prof. Tanaka, Kyoto Univ.)(5). Preliminary studies showed that with the same amount of the immobilized enzyme the apparent activity of the enzyme membrane increased with the larger and the thinner matrix. Usually 250um thickness and 3.2$cm^2$ area were employed, however, because of the operational strength and usability.

Enzyme activity of the obtained electrode was measured by a cyclic voltammetry (Ref.,SSC; Counter, Pt ; Working, 200 $mm^2$) or by following NADH and $NAD^+$ amount in the reaction mixture through HPLC method, according to the literature.(6) Cynnamaldehyde concentration was also determined by HPLC.

Results and Discussion

Figure 1 shows the cyclic voltammogram of a solution containing FcCOOH, NADH and DAP with the bare gold-plated cloth as an electrode. It is clear that the enzyme can take electrons from NADH and donate them to the electrode via mediation of FcCOOH. With use of DAP-immobilized electrode the CV-gramms could not clearly show the progress of the enzymatic reaction and therefore the reaction was followed by analyzing and quantitating the reaction product ($NAD^+$) on HPLC.

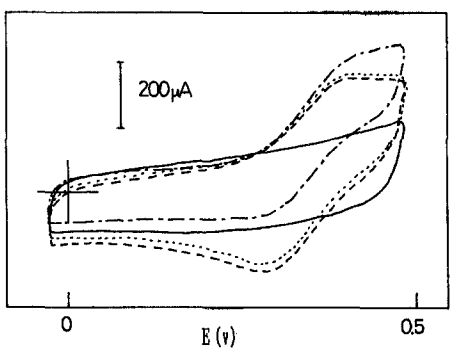



Fig 1. Cyclic voltammograms of a bare gold-plated cloth electrode in solutions containing 0.05M Tris-HCl (pH8) and 0.1M KCl (——), +0.5mM FcCOOH(------), +10 mM NADH(·····) and +20U/mL DAP(-·-·-).

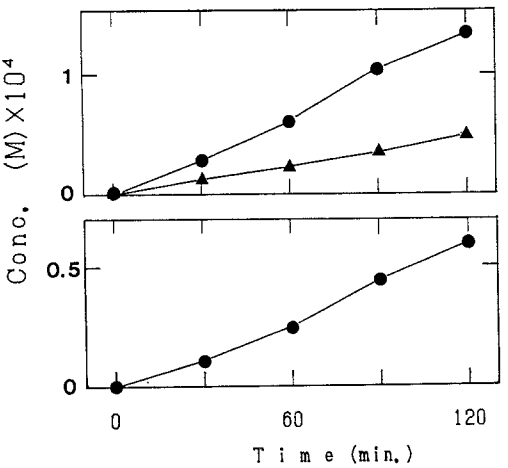



Fig.2 Electro-enzymatic oxidation of $NADH$ (a) and reduction of $NAD^+$ by DAP immobilized on gold-plated (a) or carbon-coated (b) cloth electrode.
0.1M Tris-HCl (pH 8), 0.1M KCl, 25oC.
a) 0.5V vs. SSC.
▲:2mM NADH, 0.2mM FcCOOH without DAP,
●:2mM NADH, 0.2mM FcCOOH with immobilized DAP.
b) -0.9V vs. SSC. 2mM $NAD^+$, 1mM MeV with immobilized DAP.

The time-dependent $NAD^+$ production of a reaction system containing 2mM NADH, 0.2mM FcCOOH by giving 0.5 V (vs SSC) is shown in Fig.2-a, where non-enzymatic electrode oxidation of NADH is also shown. The immobilized DAP is active, though the superiority over the non-enzymatic process is not so high. In Fig.2-b the result of $NAD^+$ reduction by use of the immobilized DAP on carbon-coated cloth electrode and MeV, as a mediator, under the constant given-voltage of -0.9 V (vs SSC) is shown. In this case also the immobilized enzyme works well and as for the reduction reaction there occurred actually no non-enzymatic process.

In Fig.3 the result for the co-immobilization of ADH and DAP is shown. Since we introduced the so-called entrapment method for the enzyme immobilization, several types of co-immobilization profiles as for the distributions of two enzymes can be conceptually prepared and among them here we tried three patterns mainly because of the simplicity in the preparations: type 1; two enzymes (DAP & ADH) were mixed with prepolymers

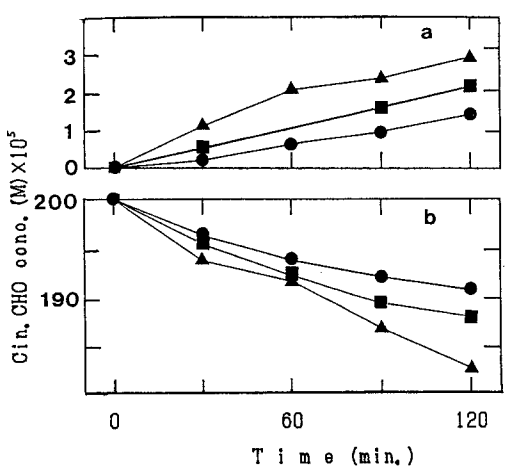



Fig.3 Electro-enzymatic production (a) and consumption (b) of trans-cinnamaldehyde by cloth electrode co-immobilizing DAP and ADH. 0.1M glycine (pH 9.6), 25°C.
a) at 0.35V vs. SSC. Substrates; cinnamyl alcohol (2.3mM), FcCOOH (0.1 mM), NADH (0.4mM). Au-Plat electrode.
▲,type 1; ■,type 2; ●,type 3
b) at -0.9V vs SSC. Substrates; trans-cinnamaldehyde (2.3mM), MeV (0.6mM), $NAD^+$ (0.4mM). Carbon-coated electrode.
Symbols are as in (a).

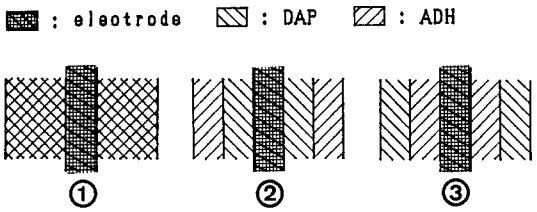



Fig.4 Three types of enzyme ditributions in enzyme-electrodes.

in one batch and then led to the membrane formation and thus practically homogeneous enzyme distributions for both enzymes will be realized; type 2, first DAP is immobilized on the electrode and then ADH-containing membrane was prepared over it and thus ADH was located mainly on the solution side of the total (two-layered) membrane and DAP was on the electrode side; type 3, the situation was inverted from type 2 (Fig.4).

The reduction of the aldehyde was followed by the decreasing concentration of the aldehyde and the oxidation reaction was with the increasing amount of it. In both reactions three types of enzyme-electrode could surely proceed the reaction and the superiority was homogeneous (type 1) > ADH on the solution side (type 2) > DAP on the solution side (type 3). As was explained in Scheme 2 the two enzyme reactions are conjugated by the coenzyme, of which migration will be the crucial process. Also the migration of electron mediator between the electrode and DAP has an important role and these two factors are to be the reasons to determine the superiority among the three distribution patterns. Actually Matsue et al., reported that only (homogeneously) mixed type could show effective current in their conjugated enzyme electrode for the oxidation of alcohol when detected by CV-gramm(7).

Thus the gold-plated or carbon-coated polyester cloth was shown to useful for the preparation of conjugated oxido-reductase electrode and the feasibility of this electrode materials will lead to the use of enzyme electrode to the various fields of reactions.

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