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Direct Bioelectrocatalysis at Metal and Carbon Electrodes Modified with Adsorbed D-Gluconate Dehydrogenase or Adsorbed Alcohol Dehydrogenase from Bacterial Membranes

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D-Gluconate dehydrogenase, a flavohemoprotein, and alcohol dehydrogenase, a quinohemoprotein, are adsorbed strongly on carbon and metal electrodes. All the electrodes with the adsorbed enzymes produce anodic currents due to the electro-enzymatic oxidation of the substrates, where the adsorbed enzymes donate electrons directly to the electrodes; neither mediators nor promoters are necessary.

Recently there have beeen considerable interest in direct electron transfer between the active center of oxidoreductases and electrodes. Thus, it has been demonstrated that promoters such as aminoglycosides are effective for accelerating the electron transfer of cytochrome c peroxidase,[1] flavohemoproteins,[2a,2b] and methylamine dehydrogenase[2c] at polished[1] and edge-oriented[2a,2b] pyrolytic graphite electrodes and modified gold electrodes.[2] It has been considered[1,2] that the promoters induce a stable enzyme-electrode interaction permitting facile electron transfer at the electrodes. In the absence of promoters, there have been only limited number of reports of the electro-enzymatic reactions due to the direct electron transfer: laccase[3], cytochrome c peroxidase[4] and methylamine dehydrogenase[2c] at an edge-oriented pyrolytic graphite electrode. We report here that D-gluconate dehydrogenase(GADH) and alcohol dehydrogenase(ADH) that are adsorbed on ordinary electrodes catalyze the electrolytic oxidation of the substrates in the absence of both promoters and electron transfer mediators.[5] No special pretreatment is necessary of the carbon and metal electrodes on which GADH or ADH is adsorbed.

GADH (EC 1.1.99.4) and ADH (no EC number) were purified from plasma membranes of *Pseudomonas fluorescene* FM-1[6a] and *Gluconobacter suboxydans*,[6b] respectively, according to the literature methods.[6] GADH

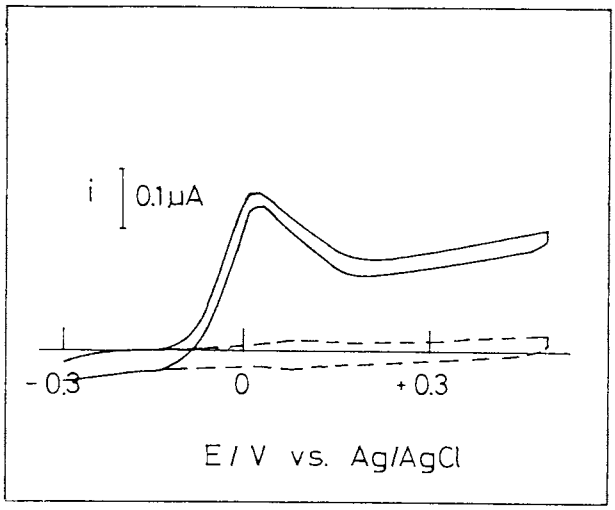

Fig. 1. Cyclic voltammograms recorded with a GADH-modified glassy carbon electrode in a pH 5.0 acetate buffer (broken line) and the buffer solution containing 20 mM GlcA (solid line) at 5 °C. Voltage scan rate: 5 mV/s.

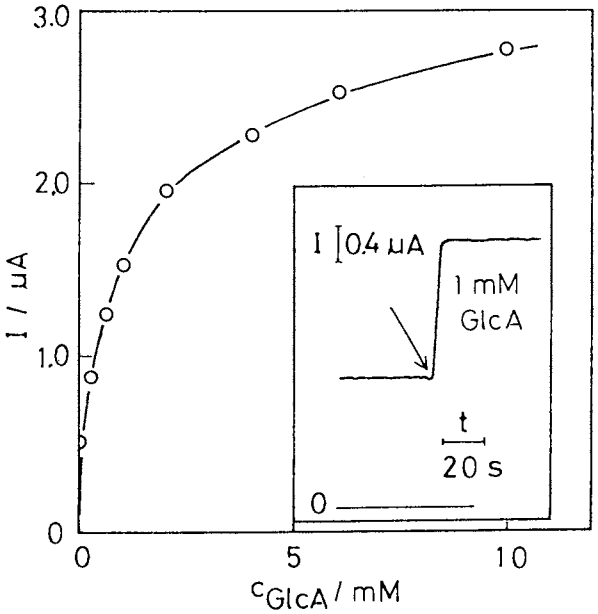

Fig. 2. Dependence of the steady state current at 0.5 V on the concentration of GlcA measured with a GADH-modified Pt-Pt electrode at 5 °C. Inset shows the current response of the electrode at 0.5 V to the addition of GlcA.

is a multiprotein complex composed of three subunits of molecular weights: 65500, 47500, and 21700, each of which contains flavin adenine dinucleotide(FAD), diheme c, and Fe-S cluster, respectively.[6a] ADH is also composed of three subunits of molecular weights, 85000, 49000, and 14400 containing pyrrolo-quinoline quinone(PQQ) and heme c, diheme c, and no redox active site, respectively.[6b] GADH and ADH were adsorbed on electrodes of glassy carbon, pyrolytic graphite, gold, platinum and silver by a dip coating method, and voltammetry was performed with a three-electrode system. Potentials were measured against an Ag/AgCl, saturated KCl reference electrode. Figure 1 shows cyclic voltammograms obtained with the glassy carbon electrode (a BAS MF-2012 GCE; 3.2 mm diameter) that was modified with adsorbed GADH, GADH being adsorbed in 1 μmol/dm^3(μM) GADH solution for 30 s followed by rinsing with pH 5.0 acetate buffer. A well-defined anodic wave was observed in the presence of the substrate, D-gluconate(GlcA). No such wave was observed at a bare glassy carbon electrode in the presence of GlcA. The wave had a shape with a hump at around +20 mV, which was similar to the wave obtained at a carbon paste electrode with adsorbed GADH.[7] Similar voltammetric waves were obtained with both edge- and basal plane pyrolytic graphite, gold and silver

electrodes that were modified with adsorbed GADH. The current magnitudes per unit geometrical surface area of the electrodes were in the same order of magnitude with these electrodes at a given potential and at a given concentration of GlcA. A current response to GlcA was also obtained with platinum and mercury electrodes when modified with adsorbed GADH though the current magnitudes were small. When a platinized platinum electrode was used, the current response was increased in magnitude; the dependence on the concentration of GlcA of the steady-state current at a GADH-modified Pt-Pt (a TOA Electronics Ltd. Pt electrode; 5 mm diameter) is shown in Fig. 2 and the response time of the electrode in the inset. The results show that GADH adsorbed on these ordinary electrodes has a catalytic activity to oxidize the subatrate electro-enzymatically by the direct electron transfer at the electrodes. It was difficult to observe the surface waves attributable to the redox reaction of the adsorbed GADH itself. The amount of the adsorbed GADH estimated as 3×10^{-12} mol/cm^2 in a monomolecular layer adsorption based on its molecular size is too small to produce the surface redox waves clearly distinguishable from the base current obtained with the bare electrodes.

ADH was also adsorbed on the ordinary electrodes to produce an anodic curret due to the electro-enzymatic oxidation of the substrate in the absence of both promoters and mediators. Figure 3 shows the voltammograms obtained with a gold-plated platinum electrode (a TOA Electronics Ltd. Pt electrode; 5 mm diameter) modified with adsorbed ADH. An anodic wave was observed in the presence of ethanol, the substrate of ADH. The voltammogram had a shape simpler than that obtained with the GADH-modified electrodes (Fig. 1). A steady-state current was attained at a given potential in seconds after the addition of ethanol to the solution. The steady-state current increased with increasing concentration of ethanol to approach a saturation value. No such current response to ethanol was observed at bare electrodes.

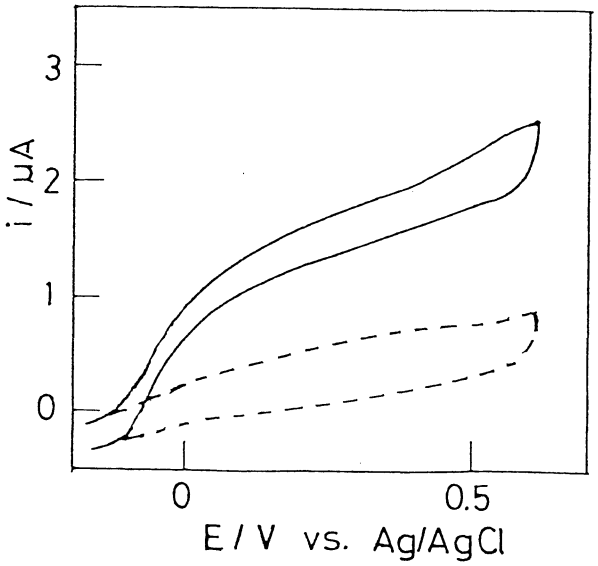

Fig. 3. Cyclic voltammograms recorded with an ADH-modified Au-Pt electrode in a pH 6.0 phosphate buffer (broken line) and the buffer solution containing 10 mM ethanol (solid line) at 5 °C. Voltage scan rate: 5 mV/s.

Both GADH and ADH contain heme c in the molecules, which are

considered to be the site to donate electrons to the respiratory chain *in vivo*.[6a,8] The heme c may be the site to react with the electrodes directly. This is in line with the observation[9] that fructose dehydrogenase(FDH), which is also a membrane-bound enzyme containing heme c, produces the catalytic currrent for the oxidation of the substarte in the absence of both promoters and mediators. However, possibility of the electron transfer reaction at the other redox sites, FAD and Fe-S cluster for GADH, and PQQ for ADH, can not be denied; different voltammetric shapes between those of GADH and ADH (Figs. 1 and 3) may suggest contributions of these redox groups to the electron transfer reaction at the electrodes. Further studies of the electro-enzymatic reactions are being pursued currently.

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