Covalent Binding of Electron Relays to Glucose Oxidase

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Modification of glucose oxidase by the covalent coupling of ferrocenecarboxylic acids gives mediator modified enzymes which undergo direct oxidation at an electrode; the use of ferroceneacetic acid instead of ferrocenecarboxylic acid is an improvement in terms of the overpotential for oxidation, the reactivity towards glucose, and the stability on storage.

There is continuing interest in the development of redox enzyme electrochemistry for applications in chemical sensors, synthesis, and bioelectronics.[1] Recently Delgani and Heller[2] reported the chemical modification of a flavoprotein which enabled direct electrochemical oxidation of the enzyme at an unmodified electrode. In their work they attached ferrocenemonocarboxylic acid to the enzyme glucose oxidase using DEC[1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride] and showed that the resulting modified enzyme could be oxidized at a platinum or carbon electrode.

Here we report preliminary results for the modification of glucose oxidase using ferrocene derivatives in which there is an alkyl chain between the cyclopentadienyl ring and the carboxylate. These modified enzymes have a number of advantages over the modification of Delgani and Heller. In particular, the use of ferroceneacetic acid gives a modified enzyme which is more stable on storage, operates at a less positive redox potential (so that the overpotential is reduced), and exhibits faster kinetics so that the catalytic glucose currents are greater.

The ferrocene modified glucose oxidase was prepared by the method described by Delgani and Heller. Samples of ferroceneacetic acid and ferrocenebutanoic acid were specially prepared. The modified enzyme was purified by gel permeation chromatography. All electrochemical measurements were carried out in deoxygenated 0.085 M phosphate buffer at pH 7.0 using a glassy carbon working electrode (4 mm diameter). The iron content of the modified enzymes was determined using atomic absorption spectroscopy.

Figure 1 shows cyclic voltammograms for the modified enzymes in the presence of sufficient glucose to saturate the enzyme kinetics. The results obtained for the ferrocenecarboxylic acid modified enzyme are in good agreement with those reported by Delgani and Heller.[2] In all cases the catalytic currents arise from mediation by ferrocenes bound to the enzyme. In control experiments, in which the enzyme was treated with the ferrocene compounds in the absence of the coupling reagent followed by gel permeation chromatography, no catalytic currents were observed.

If we assume Michaelis–Menten kinetics for the glucose

Table 1. Properties of modified glucose oxidase.

		Enzyme modifier		
	Glucose oxidase	Ferrocene-carboxylic acid	Ferrocene-acetic acid	Ferrocene-butanoic acid
$E_{1/2}$[a]/V *vs.* S.C.E.	—	0.30—0.33	0.13—0.18	0.09—0.11
No. of Fe per enzyme[b]	2	13[c]	22[d]	29[d]
k_{cat}/s^{-1}	800[e]	5[f]	1100[f]	50[f]
K_M/mmol dm^{-3}	20[g]	1[f]	5[f]	2[f]
% activity left after 1 week storage[h]	100	45	85	<10

[a] $E_{1/2}$ values (*vs.* standard calomel electrode, S.C.E.) for the free mediators are: ferrocenemonocarboxylic acid 0.290 V, ferroceneacetic acid 0.113 V, ferrocenebutanoic acid 0.095 V. [b] Determined by atomic absorption spectroscopy. [c] Delgani and Heller[2] found 14 Fe atoms per molecule of enzyme in their work. [d] This is a fraction of the groups which could undergo modification in glucose oxidase.[4] [e] At pH 10.0 in the absence of chloride.[5] [f] Calculated on the basis of the analysis in equation (1) using a value of D for glucose oxidase of 5×10^{-7} cm^2 s^{-1} and n of 2. [g] At pH 7.8.[6] [h] At 4 °C in pH 7.0, 0.085 M phosphate buffer.

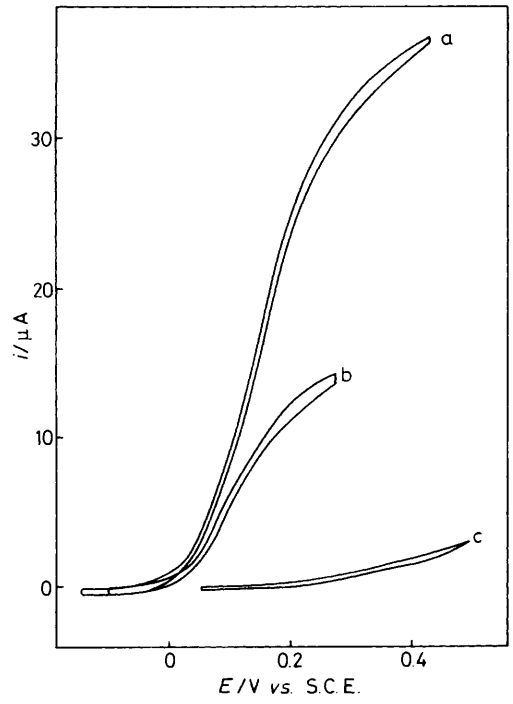

Figure 1. Voltammograms for (a) ferroceneacetic acid, (b) ferrocenebutanoic acid, and (c) ferrocenecarboxylic acid modified glucose oxidase at a glassy carbon electrode, sweep rate 5 mV s^{-1}. In all cases the concentration of glucose was sufficient to saturate the enzyme kinetics (>80 mmol dm^{-3}) and the enzyme concentration was 1 mg cm^{-3}.

$$i_L/nFA = [\text{enzyme}]\{Dk_{cat}[\text{glucose}]/(K_M + [\text{glucose}])\}^{\frac{1}{2}} \quad (1)$$

reaction we can derive expression (1) for the limiting current, i_L in the presence of glucose,[3] where D is the diffusion coefficient for the enzyme, A the electrode area, F the Faraday, and k_{cat} and K_M describe the enzyme kinetics. Figure 2 shows a plot of i_L as a function of the concentration of glucose for the modified enzyme. From the slopes and intercepts we obtain estimates of K_M and k_{cat}. These are given in Table 1 along with the $E_{1/2}$ values. From the results in Table 1 we can see that k_{cat} is considerably greater for the ferroceneacetic acid modified enzyme and that this is responsible for the increased currents we observe.

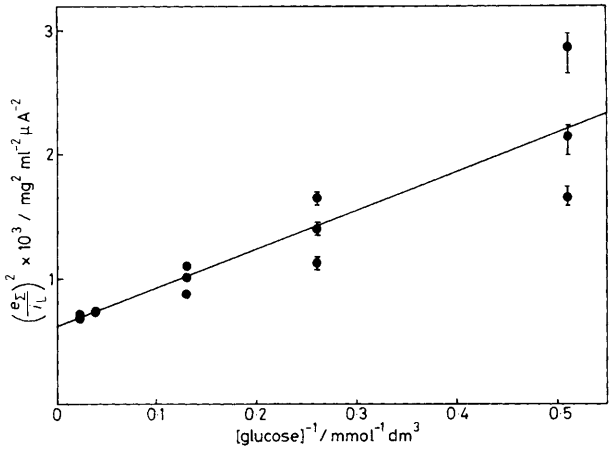

Figure 2. Plot of equation (1) for the ferroceneacetic acid modified enzyme using data for 3 different enzyme concentrations (0.45, 1.13, and 4.5 mg cm^{-3}). Similar plots were obtained for the other two cases.

We thank A. Spakovski and Dr. N. Forrow for preparing the ferrocene-acetic and -butanoic acid, and Genetics International, the S.E.R.C., and the Nuffield Foundation for financial support.

Received, 20th July 1987; Com. 1050

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