Modification of Glucose Oxidase by Tetrathiafulvalene

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Treatment of glucose oxidase with tetrathiafulvalene (TTF) in aqueous buffered solution leads to modification of the enzyme by incorporation of TTF molecules; the resulting modified enzyme undergoes direct oxidation at metallic electrodes and can be used to sense glucose.

It has been known for some time that electrodes made from conducting organic salts such as tetrathiafulvalenium tetracyanoquinodimethanide (TTF·TCNQ) can oxidise flavoproteins,[1] and a number of enzyme electrodes based upon the use of such materials have been reported.[2] However, the mechanism of the oxidation of flavoproteins at such electrodes remains the subject of dispute.[3] In this paper we present results which show that treatment of glucose oxidase (GOx) with one of the components of the organic salt, tetrathiafulvalene (TTF), leads to the modification of the enzyme and that the resulting modified enzyme can be directly oxidised at a metallic electrode.

Glucose oxidase (1.8 mg ml^{-1}) was stirred with solid TTF in pH 7.0 buffer containing 85 mmol dm^{-3} phosphate at 20 °C for 2—3 h in the dark. In some experiments 3 mol dm^{-3} urea was added to open reversibly the structure of the enzyme.[4] Undissolved solid was removed by filtration and the resulting enzyme solution was then purified by gel filtration chromatography on Sephadex G-15 eluted with 85 mmol dm^{-3} phosphate buffer pH 7.0. Saturated solutions of TTF were prepared by stirring solid TTF in the buffer followed by filtration to remove any undissolved material. All electrochemical measurements were carried out in deoxygenated phosphate buffer using a Pt or glassy-carbon disc electrode (Oxford electrodes, 0.70 cm diameter) in a conventional three-electrode cell. All potentials are reported with respect to the saturated calomel electrode (SCE).

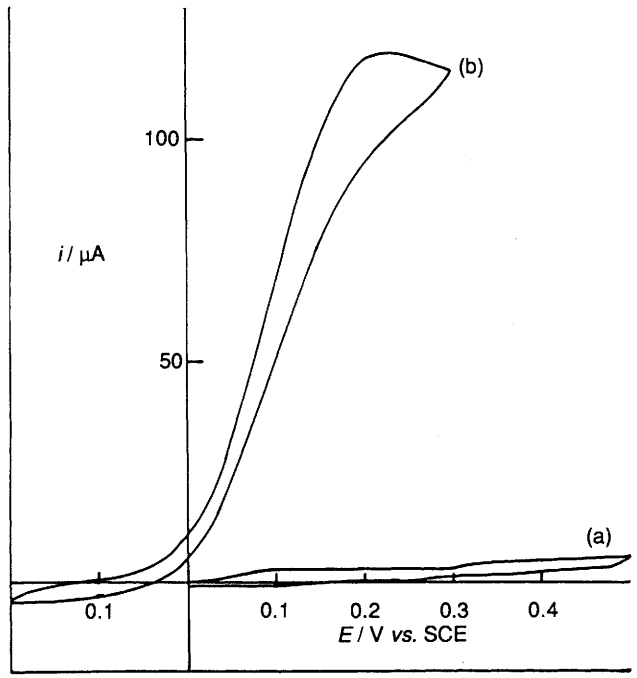

Figure 1. Cyclic voltammogram for TTF modified GOx at a platinum-disc electrode recorded at 5 mV s^{-1} in phosphate buffer pH 7.0: (a) no glucose; (b) 90 mmol dm^{-3} glucose.

Figure 1(a) shows a cyclic voltammogram for the enzyme after modification with TTF and subsequent purification. In the absence of glucose two poorly resolved waves are observed at 0.10 and 0.40 V. On the addition of glucose, Figure 1(b), a characteristic catalytic response is observed. This is consistent with the enzyme catalysed electro-oxidation of the added glucose mediated by the hydrophobic TTF molecules associated with the enzyme. No catalytic response is observed for unmodified enzyme in the absence of TTF.

It is possible that the catalytic current may arise from the mediated oxidation of glucose oxidase by TTF free in the solution. In order to test this we have compared the results for three different treatments. Figure 2(a) shows the results obtained by adding 1.8 mg ml^{-1} of solid GOx to a saturated aqueous solution of TTF. In this instance only a very small catalytic current is observed. Figure 2(b) shows the results obtained by taking a solution containing 1.8 mg ml^{-1} glucose oxidase which has been stirred with solid TTF followed by filtration. In this instance the catalytic currents are approximately ten times larger than those shown in Figure 2(a). If there were no interactions between the enzyme and the TTF molecules these two experiments should yield identical results. Finally, Figure 2(c) shows results for glucose oxidase stirred with TTF in the presence of 3 mol dm^{-3} urea and then purified by gel filtration chromatography. In this instance the catalytic currents are approximately five times greater than those shown in Figure 2(a), despite the fact that the gel filtration chromatography efficiently separates the high molecular weight enzyme from free TTF in solution.

We have carried out similar experiments using solid TTF·TCNQ in place of TTF. Again we observe catalytic currents for enzyme that has been stirred with the solid and then purified, however, the catalytic currents are less reproducible in this instance and this is the subject of further study.

Our results indicate that GOx can be modified by the incorporation of a hydrophobic redox mediator and that the resulting modified enzyme is electroactive. In this sense the

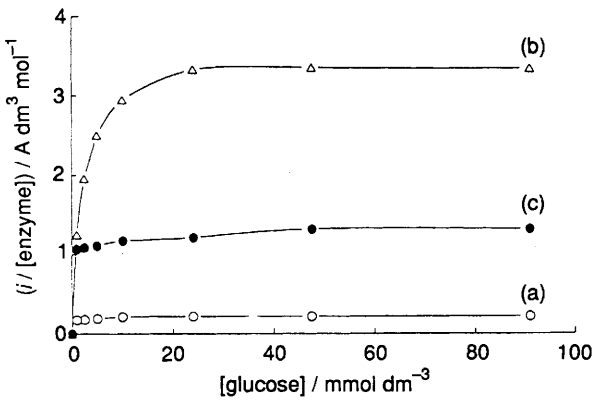

Figure 2. Plot of the catalytic response as a function of the concentration of added glucose: (a) 1.8 mg ml^{-1} GOx added to a saturated solution of TTF in buffer; (b) 1.8 mg ml^{-1} GOx stirred with solid TTF; (c) as (b) but after purification by gel filtration chromatography.

results are similar to those reported for the covalent modification of GOx with ferrocenyl groups.[4] These observations may, in part, explain the observed electrochemistry of glucose oxidase and other flavoproteins at conducting salt electrodes.

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