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Enzyme Electrocatalysis at the TTF-TCNQ Electrode [and Discussion]

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Enzyme electrocatalysis at the TTF–TCNQ electrode

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We provide a chemical basis for the unusual properties of the glucose oxidase/TTF–TCNQ enzyme electrode. This permits an explanation of the behaviour of many organic salt systems, and provides a framework for designing an electrode material for a given application.

Introduction

The conducting organic salt tetrathiafulvalene-tetracyanoquinodimethane (TTF–TCNQ) has been of great interest to solid-state physicists and physical chemists since its synthesis in 1973 (Ferraris 1973). The first use of this material as an electrode was described some years later (Jaeger & Bard 1979), but the analytical utility of conducting salts was only demonstrated when Kulys achieved the biocatalytic oxidation of glucose on unmodified organic metal electrodes (Kulys *et al.* 1980). Subsequent work (Albery *et al.* 1985, 1987; Hale & Wightman 1988; McKenna & Brajter-Toth 1987) illustrated the broad applicability of TTF–TCNQ to bioanalysis.

The unique chemical and structural properties of TTF–TCNQ have been discussed at length (Alcacer 1980; Miller & Epstein 1978; Wudl 1984; Torrance 1978). These studies also provide insight into the possible structure of the electrode-solution interface. The anisotropic nature of the material would suggest that a polycrystalline electrode should exhibit microscopic heterogeneity, which complicates attempts to model the behaviour of the electrode. Orbital overlap along the *b*-axis of a TTF–TCNQ crystal, for example, is much larger than along other axes (Torrance 1978). It might be reasonably expected, therefore, that electron transfer reactions could proceed at a higher rate along this axis and that a macroscopic electrode would exhibit a distribution of heterogeneous rate constants, depending on the nature and site of the electrode–analyte interaction. Similarly, the energetics for adsorption of solution species could vary as a function of the crystal face exposed at the interface. Since the extent of charge transfer between TTF and TCNQ has been shown to be a function of solvent polarity (Tomkiewicz *et al.* 1974), the species present at the surface of an electrode may also be affected by the local solvent dielectric constant. Perhaps most significant, however, is the fact that the electrode material can undergo faradaic reactions at potentials of analytical interest (table 1).

As we reported previously (Hill *et al.* 1988), the enzyme electrode exhibits an anomalous rotation speed dependence indicative of mediator production at the electrode surface. Other observations which must be addressed by any model of the system include the presence of an adsorbed enzyme layer which is involved in the

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Table 1. *Potentials for organic salt redox processes*

reaction	peak potential (V against SCE)	ref.
$TTF^{+} + TCNQ^{0} + e^{-} \leftrightarrow TTF\text{–}TCNQ$	+0.71[c]	Jaeger & Bard (1979)
$TTF^{2+} + e^{-} \leftrightarrow TTF^{+}$	+0.66[a]	Coffen *et al.* (1971)
$TTF^{0} + Cl^{-} \rightarrow TTFCl + e^{-}$	+0.53	Jaeger & Bard (1979)
$NaTCNQ \rightarrow TCNQ^{0} + Na^{+} + e^{-}$	+0.39	Jaeger & Bard (1979)
$KTCNQ \rightarrow TCNQ^{0} + K^{+} + e^{-}$	+0.30	Jaeger & Bard (1979)
$TTF^{+} + e^{-} \leftrightarrow TTF^{0}$	+0.30[a]	Coffen *et al.* (1971)
$TCNQ^{0} + e^{-} \leftrightarrow TCNQ^{-}$	+0.12[b]	Sharp (1976)
$TTFCl + e^{-} \rightarrow TTF^{0} + Cl^{-}$	+0.01	Jaeger & Bard (1971)
$TCNQ^{0} + K^{+} + e^{-} \rightarrow KTCNQ$	−0.05	Jaeger & Bard (1971)
$TCNQ^{0} + Na^{+} + e^{-} \rightarrow NaTCNQ$	−0.05	Jaeger & Bard (1971)
$TCNQ^{-} + e^{-} \leftrightarrow TCNQ^{2-}$	−0.13	Sharp (1976)
$TTF\text{–}TCNQ + e^{-} \leftrightarrow TTF^{0} + TCNQ^{-}$	−0.27[c]	Jaeger & Bard (1971)

[a] Acetonitrile/TEAP solution. [b] Acetonitrile/$LiClO_4$ solution. [c] Calculated.

electrocatalytic reaction and the absence of a clear mediator wave in cyclic voltammograms. Detailed understanding of the TTF–TCNQ enzyme electrode will clearly require identifying and characterizing the species present at the interface.

Experimental

The TTF–TCNQ electrodes and the instrumentation used for electrochemical measurements have been previously described (Hill *et al.* 1988). All compounds were reagent grade and used as received, unless otherwise specified. Synthesis of TTF–TCNQ and radiolabelled enzyme has been detailed elsewhere (Hill *et al.* 1988). $TTFClO_4$ was synthesized by the method of Hunig (Hunig *et al.* 1973) and LiTCNQ produced as described by Melby (Melby *et al.* 1962). All electrochemical measurements were performed under an argon atmosphere unless otherwise noted. Mass spectral data were acquired at the Mass Spectrometry Laboratory at the University of Kansas.

Results and discussion

In spite of previous reports of the formation of insoluble TTF salts (Jaeger & Bard 1979), we find that TTF^{+} has a solubility of the order of 0.1 mM in phosphate-buffered saline solution. The cyclic voltammogram is much less reversible than in acetonitrile (figure 1). It is noteworthy that both TTF and TCNQ have redox potentials well positive of that for glucose oxidase (*ca.* −0.3 V against SCE) (Stankovich *et al.* 1978), and that TCNQ has been shown to be an efficient mediator for glucose oxidase (Cenas & Kulys 1980). The solubility of TTF^{+} thus immediately suggests the possibility of mediated electron transfer by a soluble electrode species. Indeed, the addition of $TTFClO_4$ to a solution of reduced enzyme causes an increase in the anodic current. Furthermore, the presence of the soluble redox species changes the rotation speed dependence of the system. Whereas in the absence of added mediator the current decreases as the rotation speed increases, the current increases with rotation speed in the presence of soluble TTF^{+}, in accordance with the Levich equation. This is perfectly understandable if the TTF^{+} is being generated at the electrode surface in the first case (with a bulk concentration near zero) and is supplied to the interface from solution in the second.

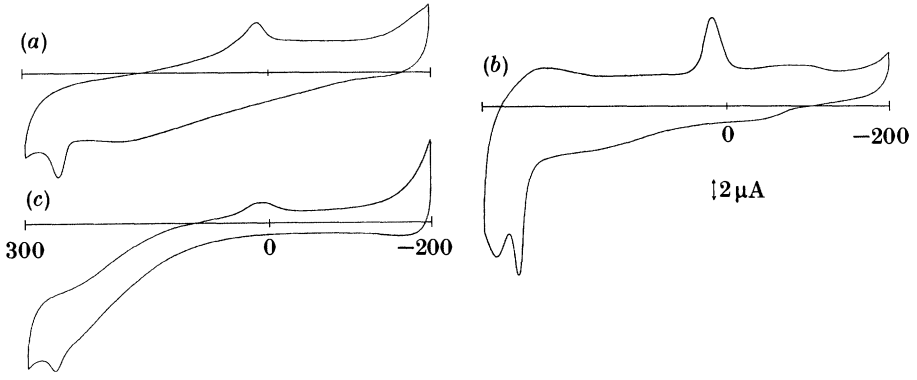

Figure 1. Cyclic voltammetry at the TTF–TCNQ electrode. Scan rate of 10 mV s^{-1}, stationary electrode of 0.164 cm^2 geometric area under argon atmosphere. (*a*) Blank scan in phosphate-buffered saline, pH 7.4 (PBS); (*b*) 0.2 mM TTF–ClO_4 in PBS; (*c*) PBS, 100 mM in glucose, with 1.4 μM glucose oxidase.

Further support for this hypothesis comes from the fact that addition of LiTCNQ to the enzyme solution *decreases* the anodic current. Although $TCNQ^0$ can behave as a mediator, addition of $TCNQ^-$ will cause a decrease in the concentration of TTF^+ due to formation of insoluble TTF–TCNQ. The extraordinarily low solubility of $TCNQ^0$ (and the sodium and potassium salts of the anion) essentially constrains it to the interface. The cyclic voltammogram of LiTCNQ in lithium acetate electrolyte bears a striking resemblance to that of the enzyme electrode. The shape of this wave is likely due to the modification of the electrode surface by the insoluble $TCNQ^0$, since the same behaviour is observed on a platinum electrode. We can therefore envision two independent modes of electron transfer: a homogeneous reaction between the reduced enzyme and TTF^+ followed by heterogeneous electron transfer from TTF^0, and a heterogeneous reaction between enzyme and $TCNQ^0$ on the electrode surface. The flux through each pathway will be a function of the concentration of the species involved. At low potentials, very little TTF^+ will be generated and the $TCNQ^0$ is expected to provide the major path for electron transfer; at more positive potentials the TTF^+ pathway can become more important and at +300 mV it dominates the electrode behaviour. Note that the TTF^+ pathway is much more efficient, as observed in previous work (Cenas & Kulys 1980).

The evidence for the role of the inactive, adsorbed enzyme layer in bio-electrocatalysis has been presented previously (Hill *et al.* 1988). Briefly, the rapid recycling of soluble enzyme due to the reaction with substrate should result in an enhanced current proportional to $(k_{cat})^{\frac{1}{2}}$ because the thickness of the reaction layer is a function of the enzyme kinetics and the concentrations of enzyme and substrate (Albery *et al.* 1985). Thus at saturation the maximum expected current density would be equal to $nF(Dk_{cat})^{\frac{1}{2}}$ [GOx]. Assuming a diffusion coefficient of 4×10^{-7} cm^2 s^{-1} for the enzyme and a value of k_{cat} of 500 s^{-1}, a saturation current density of less than 4 μA cm^{-2} is expected for a one micromolar enzyme solution. The observed current density is, however, over 20 μA cm^{-2} (Albery *et al.* 1985; Hill *et al.* 1988). Furthermore, the expected response when the enzyme is below substrate saturating levels should be proportional to the square root of the glucose concentration. This is not observed. In essence, neither the magnitude nor the functional dependence of the current can be explained by models based on freely

diffusing enzyme, suggesting the presence of a reversible adsorption process (Hill *et al.* 1988).

The limited lifetime of these electrodes can be understood in terms of loss of mediator from the surface. Collection of buffer after extended cycling of electrodes in air and extraction into chloroform yields a trace amount of yellow solid from the yellowish solution that is produced. Mass spectral analysis of the solid reveals that it is TTF or a TTF salt, confirming the previous arguments. The surface of the electrode is covered with a yellow film which is probably $TCNQ^0$. TCNQ is not detected in the buffer.

It has been further observed that soaking an enzyme-coated electrode which has lost its activity in an FAD solution (1–2 mM) can often regenerate up to 10% of the original response. Finally, adsorption of apoenzyme to the electrode before measuring the response to soluble reduced enzyme results in a dramatic loss of sensitivity (up to 80%). This implies that either the structure of the adsorbed layer is influenced by the presence of FAD or that FAD is a participant in the electron transfer process. The role of FAD in maintaining the structure of the holoenzyme is well established (Swoboda 1969), but the situation may be quite different upon adsorption. Another potential role for the adsorbed enzyme layer is to trap mediator species at the interface. This may reduce the rate of loss of electrode surface components, thereby extending the lifetime and increasing the efficiency of the system. Preliminary experiments indicate that $TCNQ^0$ can form covalent adducts with the enzyme. Due to the high surface concentration of both species, this may result in significant modification of the adsorbed layer. It is not yet established whether TTF can undergo similar reactions.

Conclusions

Although still incomplete, we believe that our studies present a model which can both explain many of the confusing aspects of these enzyme electrodes and also provide a framework for understanding the chemical processes involved. For example, the formation of a *conducting* solid between TCNQ and a donor places certain constraints on the redox potential of the donor (Torrance 1978). Thus it is not surprising that many of the organic metals exhibit bioelectrocatalytic behaviour with enzymes of relatively low redox potentials. In fact, one would qualitatively predict that as the donor potential of the salt becomes more positive, the rate of the mediated reaction (and hence the current density at the electrode) would increase. (This is so because the mediator reaction is rate limiting, a fact clearly demonstrated by the increased current observed upon addition of $TTFClO_4$.) This is in accordance with experimental results (Albery *et al.* 1985). The retention of $TCNQ^0$ on the electrode interface due to its insolubility permits the accumulation of negative charge on such electrodes (Cenas & Kulys 1980) when reduced enzyme is present. Finally, the presence of simultaneous mediated mechanisms coupled with the electrode heterogeneity and the poor solubility of several of the electrode components clarifies the absence of a mediator wave in cyclic voltammetry. We feel that this work allows the unique behaviour of organic salt enzyme electrodes to be placed in a chemical context, permitting better understanding of the system studied. In addition, by considering the solubility and redox properties of the donor species, one may be able to design an electrode for a given analytical application. By careful selection of the redox potential of the donor, the selectivity of the electrode may be modified. Similarly, a balance between sensitivity and electrode lifetime should be

possible by adjusting the solubility of the donor. Thus the insight generated from the study of the chemical properties of these organic salt electrodes will be extremely useful to bioelectrochemists in the future.

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Discussion

P. N. Bartlett (*University of Warwick, U.K.*). I think that Professor Wilson may be correct when he says that there may be more than one mechanism in operation at the TTF–TCNQ electrode surface. In recent studies at Warwick (described on our poster) we have shown that TTF can be used to 'modify' glucose oxidase and that the resulting 'modified' enzyme undergoes direct oxidation at platinum or glassy carbon electrodes at potentials very similar to those shown by Professor Wilson for glucose oxidase oxidation at a TTF–TCNQ electrode. Our results suggest that the TTF molecules are incorporated into hydrophobic regions within the protein and that they can then act as mediators for the electron transport into the flavin active site.

In our experience the single crystal studies give the best results for mechanistic studies because there is no surface roughness problem and because of the much lower background currents. Has Professor Wilson seen any evidence for adsorbed glucose oxidase on single crystal TTF–TCNQ electrodes?

G. S. Wilson. Yes, the use of radiolabelled glucose oxidase shows unequivocal adsorption on single crystals of the same order of magnitude as on pressed pellets or sublimed TTF–TCNQ. It is not clear, however, that the use of single crystals obviates the need to consider surface roughness. The difficulties associated with growing crystals of TTF–TCNQ are well documented, and it is quite common to produce crystals with highly fractured surfaces.

W. J. Albery (*University of Oxford, U.K.*). In our work and work carried out by Dr Bartlett we find that the order of the reaction with respect to enzyme concentration is one half. We have carried out these experiments over a wide enough concentration range to be able to discriminate between the square root dependence and the similar curvature produced by a Langmuir isotherm. How does Professor Wilson's proposed mechanism account for the half-order dependence? Furthermore if the inverse rotation speed dependence is to be explained by the rotation speed controlling the surface concentration of the mediator, then the flux of mediator escaping into the bulk solution must be comparable or larger than the flux involved in enzyme turnover. Our ring-disc experiments show that the concentrations of electroactive compounds in the diffusion layer are much smaller than the concentration required to explain the inverse rotation speed dependence by his mechanism.

G. S. Wilson. In our experience the order of the current response with respect to reduced enzyme concentration is not a simple half-order relationship. At low enzyme concentrations (below about 0.5 μM), the current exhibits a sigmoidal correlation suggestive of an adsorption isotherm. At higher concentrations the response is half-order. We interpret this as being due to adsorption of soluble, active enzyme on a surface which has been first modified by the irreversibly bound inactive enzyme. The half-order dependence is observed as those sites become saturated for the reasons you have suggested for a surface-controlled reaction.

The issue of the mediator flux is an important one. If the entire current were due to a soluble species, for example, a flux of about 10^{-9} mol cm^{-2} s^{-1} would be required. If the limiting decrease in current resulting from the rotation of the electrode is ascribed solely to mediator loss, then this flux should exceed 10^{-10} mol cm^{-2} s^{-1}. This, of course, should lead to microamperes of current at the ring if an electroactive species could be detected. Why do we not see such currents? Such a rate of dissolution of the electrode to produce soluble mediator would correspond to the loss of about six equivalent monolayers per minute. This is clearly impossible because total loss of adsorbed enzyme in a matter of minutes would be predicted and this is not observed in the radiochemical experiments. Our rough estimates of the rate of dissolution of the electrode to form soluble TTF^+ would yield a maximum flux of 10^{-12} mol cm^{-2} s^{-1} and a maximum ring current of less than 50 nA. We cannot detect such currents with our RDE system but if we assume a catalytic enhancement of 400, then this flux would support the disc current we see. The overall process is therefore supported primarily by species which do not move very far from the electrode surface. If TTF^+ is reduced by incoming enzyme, then it would be converted into insoluble TTF^0, thus entrapping it. This is another reason why TTF^+ cannot be detected under reducing conditions. We think that the TTF^+ can come off the electrode and pass through the adsorbed enzyme layers. It may also be trapped in these layers and recycled. At very high concentrations of enzyme (greater than 20 μM) the anodic current actually begins to decrease and an unstable response results. This may be due to a decrease

in the availability of mobile, soluble mediator which cannot interact with the incoming enzyme. Thus how far a mediator can escape from the electrode surface may depend on redox trapping by the reduced enzyme. It is further possible that TTF is carried away from the surface entrapped in the soluble enzyme. Under the conditions of our experiments, a loss of about 50 equivalent monolayers of TTF could produce enough material to react with soluble enzyme in a 1:1 ratio. Although the mediator may have to diffuse through adsorbed inactive enzyme this is still not unreasonable. The dependence of current on rotation speed is neither simple inverse (w^{-1}) or inverse square root suggesting a mixed mechanism involving both soluble and bound species. We think that our model qualitatively meets these requirements.

W. J. Albery. I am interested in the question of the roughness factors and the fact that excluding a roughness factor 20 layers of inactive enzyme would be found. The scanning electron microscope picture of Professor Wilson's polycrystalline electrode looks very similar to the ones we obtain for our polycrystalline electrodes. The crystallites and fjords are on the micrometre scale. The size of the enzyme molecule is several orders of magnitude smaller than this and therefore in my view must be able to penetrate the fjords. Hence a polycrystalline electrode must have a substantial roughness factor. Indeed we and Dr Bartlett find that a polycrystalline electrode has a much larger double layer capacity compared with an electrode made of a pressed pellet or of a single crystal. I expect that the electrochemical measurements of roughness that gave results close to unity have not got sufficient time resolution to penetrate the fjords. With distance of the order of 10^{-4} cm and $D \approx 10^{-5}$ cm^2 s^{-1} the associated time is 1 ms which is much less than the double layer charging time. Hence I conclude that the electrochemical measurements do not measure the true roughness and that the number of layers of inactive enzyme is one or less.

G. S. Wilson. In this case the determination of the roughness factor of the organic salt surfaces is extremely difficult. For this reason we have chosen to express the surface coverage of enzyme as 'equivalent monolayers'. Since we have used the geometric area for this calculation, the reported coverage represents the maximum coverage in monolayers that could be achieved. Since the roughness factor is certainly greater than unity, this means that the actual coverage could well be only a monolayer. We would be very happy with this conclusion since it is then not necessary to explain how electron transfer can occur through multilayers of enzyme. Our arguments concerning the mechanism of action of the enzyme do not depend, however, on knowing the exact coverage. Our radiochemical and other experiments (Hill *et al.* 1988) make it absolutely clear in our case that adsorbed and inactive enzyme is necessary for proper functioning of the sensor. Such a conclusion does not in any way preclude reversible adsorption of enzyme in addition. In fact we view it as absolutely necessary.

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