Enantioselective bioelectrocatalyzed oxidation of glucose by glucose oxidase at chiral-monolayer-electrodes

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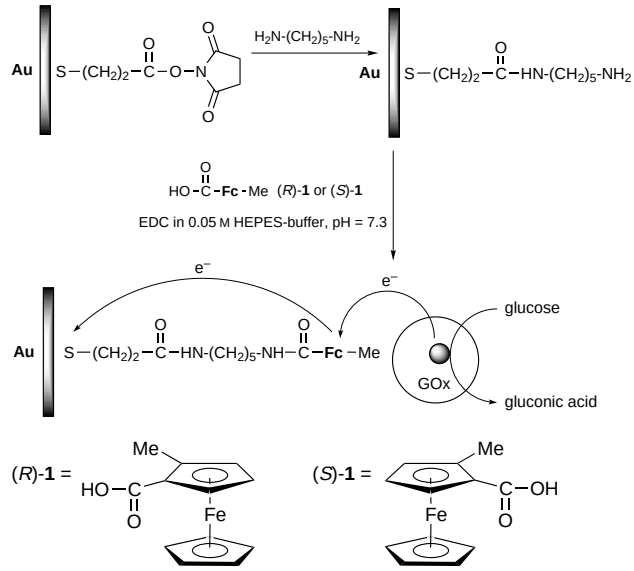
(*R*)- or (*S*)-2-Methylferrocenecarboxylic acid monolayers assembled onto Au-electrodes stimulate chiroselective bioelectrocatalysed oxidation of glucose in the presence of glucose oxidase

Chiral discrimination in electron transfer or energy transfer processes in biological assemblies has been a subject of extensive research.[1,2] Chiroselectivity was observed in the association of optically-active transition metal complexes to DNA[3] and enantioselective electron transfer in the DNA microenvironment was reported.[4] Chiroselective electron transfer between transition metal complexes and redox proteins such as plastocyanine,[5] cytochrome c,[6] plant ferredoxin[7] and reconstituted Zn^{II}-myoglobin[8] was reported, although in low discrimination yields. Mediated electron transfer between low molecular-weight redox-relays and redox proteins has been studied in the general context of electrochemical biosensors.[9,10] In particular, ferrocenyl cation-mediated[11] oxidation of oxidoreductases was extensively explored, and the mediated electron transfer rates were controlled by the ferrocene substituent groups and the redox potentials of the relays. As the association rate of ligands to proteins is lower than diffusion-controlled (*k ca.* 10^7–10^8 M^{-1} s^{-1}) it is anticipated that chiral discrimination could play an important role in the affinity interactions of ligands and proteins. Indeed, we have reported the first example of enantioselective, diffusionally mediated, bioelectrocatalysed oxidation of glucose in the presence of chiral ferrocene mediators.[12] These results were later criticized by Alzari *et al.*,[13] though recent examples by Sadeghi and co-workers[14] nicely support the concept of chiroselective, diffusionally mediated, electron transfer within enzymes. Mediated bioelectrocatalytic transformations at redox-relay functionalized monolayer electrodes was recently exemplified.[15] Chiral monolayer-modified electrodes were developed for some electrochemical,[16] but not bioelectrochemical applications. Here we report on the assembly of chiral monolayers consisting of (+)-2-methylferrocene carboxylic acid, (*R*)-**1** and (−)-2-methylferrocenecarboxylic acid, (*S*)-**1**.[17] The resulting chiral monolayer interfaces stimulate the enantioselective mediated bioelectrocatalysed oxidation of glucose. Mechanistic analysis of the electron transfer process using a rotating disc electrode (RDE), reveals chiral discrimination in the affinity associative interactions between the chiral monolayer and the redox enzyme glucose oxidase (from *Aspergillus niger* type X-S, E.C. 1.1.2.4, Sigma).

The assembly of the amide-functionalized chiral ferrocene monolayers on Au-electrodes is shown in Scheme 1. The ferrocene monolayer-functionalized electrodes exhibit a reversible cyclic voltammogram, E° = 0.235 V *vs.* SCE, and the surface coverage of the two enantiomers is similar, 6×10^{-11} mol cm^{-2} (±2%). The electron transfer rates to the two chiral ferrocene relays was determined by Laviron's method[18] and were found to be identical, k_{et} *ca.* 18.0 s^{-1}. Fig. 1 shows the cyclic voltammograms of the chiral ferrocene-functionalized electrodes, in the presence of glucose oxidase, GOx, in the electrolyte solution in the absence of glucose [curves (*a*) and (*b*)] and in the presence of added glucose, 5×10^{-2} M [curves (*c*) and (*d*)]. In the presence of glucose electrocatalytic anodic currents are observed in the presence of the two redox-functionalized electrodes. The electrocatalytic anodic current in the presence of the (*S*)-**1**-functionalized monolayer is higher by *ca.* 90% as compared to the electrocatalytic current in the presence of the (*R*)-**1**-functionalized monolayer electrode. These results clearly demonstrate that chiroselective bioelectrocatalysed oxidation of glucose occurs at the functionalized electrodes.

To understand the origin of chiroselective mediated electron transfer at the chiral monolayer electrodes, the bioelectro-



Scheme 1 Organization of the chiral ferrocene monolayer-modified Au-electrodes and their electrochemical interaction with reduced glucose oxidase, GOx^{red}

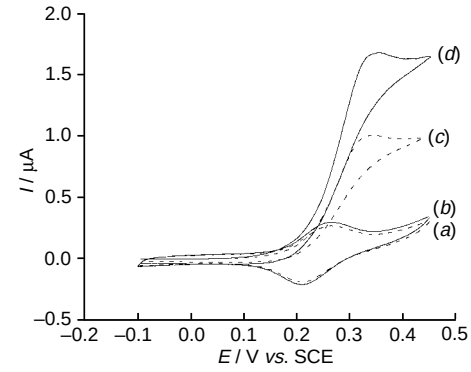


Fig. 1 Cyclic voltammograms of the chiral ferrocene monolayer-modified Au-electrodes (0.5 mm diameter Au-wire, 0.2 cm^2 geometrical area, roughness coefficient, *ca.* 1.3); (*a*) and (*b*) (*S*)-**1** and (*R*)-**1** monolayer-modified electrodes, respectively, in the presence of 1×10^{-5} M GOx and in the absence of glucose; (*c*) and (*d*) (*R*)-**1** and (*S*)-**1** monolayer-modified electrodes, respectively, in the presence of 1×10^{-5} M GOx and 50 mM glucose. Background electrolyte, 0.1 M phosphate buffer, pH 7.0. Potential scan rate, 5 mV s^{-1}.

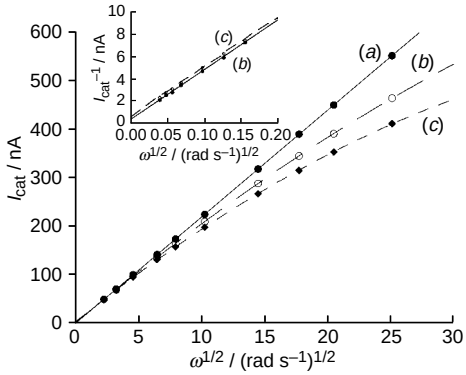


Fig. 2 Currents generated at the chiral ferrocene monolayer-modified Au-electrodes (Au-disk, 0.16 cm² geometrical area, roughness coefficient, *ca.* 1.3) in the presence of 1×10^{-5} M GOx and 5×10^{-5} M glucose at applied potential +0.4 V *vs.* SCE. (*a*) Theoretical Levich plot, (*b*) **(*S*)-1** monolayer-modified electrode, (*c*) **(*R*)-1** monolayer-modified electrode. Background electrolyte, 0.1 M phosphate buffer, pH 7.0. Inset: Koutecky–Levich plots for the experimental results shown.

catalysed process was examined at a rotating disc electrode (RDE).[19] The two chiral ferrocenes, (*R*)-**1** and (*S*)-**1** were assembled on an Au-disc electrode as described in Scheme 1, and the resulting catalytic currents were monitored at a low glucose concentration, 5×10^{-5} M, and under Ar in order to preserve the flavo enzyme redox centres in their reduced states, GOx^{red}. Fig. 2 (*b*) and (*c*) shows the experimental Levich plots for the electrocatalysed oxidation of GOx^{red} by the (*S*)-**1** and (*R*)-**1** monolayers, respectively. For comparison, the theoretical Levich plot for the oxidation of GOx^{red} by the ferrocene monolayer is shown in curve (*a*) (assuming the transfer of 2 electrons, $n = 2$; and the diffusion coefficient $D_{GOx} = 4 \times 10^{-7}$ cm² s^{-1}). The inset in Fig. 2 shows the related Koutecky–Levich plots for the electrocatalysed oxidation of GOx^{red} by (*R*)-**1** and (*S*)-**1** monolayers, respectively. The slopes of the two plots are identical and correspond to the transfer of two-electrons (Fig. 2, inset). Thus, even though GOx consists of a dimer and the oxidation of each of the FAD sites in the subunits involves two electrons, the transfer of only two electrons is observed in the RDE experiments. This is rationalized by the fact that only one subunit is appropriately aligned in respect to the monolayer within the electron transfer event. It should be noted that in the RDE experiments, analysing the electron transfer rate between the chiral ferrocene monolayers and GOx, a *low* concentration of glucose was used under anaerobic conditions. The use of a low glucose concentration is essential to derive the electron transfer rate constant from reduced GOx, GOx^{red}, to oxidized ferrocene under conditions where the regeneration of GOx^{red} by glucose is impossible (very slow) during monolayer and enzyme interaction time. From the intercepts of the respective Koutecky–Levich plots, the electrochemical electron transfer rates from the (*R*)-**1** and (*S*)-**1** monolayer were derived to be 0.51×10^{-2} and 0.9×10^{-2} cm s^{-1}, where the overall rate constants ($k_{overall} = k_{el}/\Gamma_{ferrocene}$, where $\Gamma_{ferrocene}$ is the surface coverage of the electron mediator) correspond to 0.85×10^5 and 1.5×10^5 M^{-1} s^{-1}, respectively. The overall electron transfer rates for the oxidation of GOx by the two chiral monolayer-electrodes depend inversely on the concentration of GOx. This indicates that the electrocatalysed oxidation of GOx by the ferrocene monolayer proceeds via the formation of a complex between the monolayer and GOx^{red}, rather than by outer sphere electron transfer,[19] Scheme 2.

$$\left|-Fc^{+} + GOx^{red} \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}}\right| -Fc^{+} \cdots GOx^{red} \xrightarrow{k_{+2}} \left|-Fc + GOx^{ox}\right.$$

Scheme 2

Following Scheme 2, the overall rate constant is given by eqn. (1), where K_M is given by eqn. (2). We find that the electron transfer rates from the GOx^{red} to the (*R*)-**1** and (*S*)-**1** monolayer differ substantially, k_{+2}(*R*)-**1** = 0.3 s^{-1}, k_{+2}(*S*)-**1** = 0.5 s^{-1}, while the K_M values characterizing the chiral monolayer and GOx^{red} are identical, $K_M = 1.2 \times 10^{-5}$ M.

$$k_{overall} = k_{+2}/(K_M + [GOx^{red}]) \quad (1)$$

$$K_M = (k_{-1} + k_{+2})/k_{+1} \quad (2)$$

We thus conclude that the affinities for the association of GOx^{red} to the chiral ferrocenyl cation monolayer are similar. The electron transfer rates between the reduced flavin centre and the chiral ferrocenyl cations differ substantially. The diastereoisomeric nature of the complexes between GOx^{red} and the chiral ferrocenyl cations could yield different electron transfer distances to the flavin sites, thereby stimulating the enantioselective bioelectrocatalysed oxidation of glucose.

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Footnote and References

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1 L. Casella and A. Marchesini, *Biochemistry* 1994, **33**, 6377; R. A. Marushak, T. A. Shields and A. G. Lappin, in *Electron Transfer in Biology and the Solid State*, ed. M. K. Johnson, R. B. King, D. M. Kurtz, Jr., C. Kutal, M. L. Norton and R. A. Scott, ACS Advances in Chemistry 226, American Chemical Society, Washington, DC, 1990, p. 237.
2 A. Fersht, *Enzyme Structure and Function*, Freeman WH and Co., New York, 2nd edn., 1984, pp. 221–247.
3 A. M. Pyle, T. Morii and J. K. Barton, *J. Am. Chem. Soc.* 1990, **112**, 9432; N. J. Turro, J. K. Barton and D. A. Tomalia, *Acc. Chem. Res.* 1991, **24**, 332.
4 M. D. Purugganan, C. V. Kumar, N. J. Turro and J. K. Barton, *Science* 1988, **241**, 1970.
5 K. Bernauer and J.-J. Sauvain, *J. Chem. Soc., Chem. Commun.*, 1988, 353; J. R. Pladziewicz, M. A. Accola, P. Osvath and A. M. Sargeson, *Inorg. Chem.*, 1993, **32**, 2525.
6 S. Sakaki, J. Nishijima, H. Koga and K. Ohkubo, *Inorg. Chem.*, 1989, **28**, 4063; S. Sakaki, J. Nishijima and K. Ohkubo, *J. Chem. Soc., Dalton Trans.*, 1991, 1143; S. J. Meskers, M. Ubbink, G. W. Canters and H. P. J. M. Dekkers, *J. Phys. Chem.*, 1996, **100**, 17 957.
7 K. Bernauer, M. Monzione, P. Schürmann and V. Viette, *Helv. Chim. Acta*, 1990, **73**, 346; K. Bernauer, in *Electron-Transfer Reactions*, in *Metalloproteins, Metal Ions in Biological Systems*, 27, ed. H. Sigel and A. Sigel, Marcel Dekker, New York, 1991, p. 265.
8 K. Tsukahara, C. Kimura, J. Kaneko and T. Hara, *Chem. Lett.*, 1994, 2377.
9 P. N. Bartlett, P. Tebbutt and R. G. Whitaker, *Prog. React. Kinet.*, 1991, **16**, 55.
10 W. Schuhmann and H.-L. Schmidt, in *Advances in Biosensors*, ed. A. P. F. Turner, JAI, London, 1992, vol. 2, p. 79.
11 A. E. G. Cass, G. Davis, M. J. Green and H. A. O. Hill, *J. Electroanal. Chem.*, 1985, **190**, 117.
12 S. Marx-Tibbon, E. Katz and I. Willner, *J. Am. Chem. Soc.*, 1995, **117**, 9925.
13 P. Alzari, N. Anicet, C. Bourdillon, J. Moiroux and J.-M. Savéant, *J. Am. Chem. Soc.*, 1996, **118**, 6788.
14 S. J. Sadeghi, G. Gilardi, G. Nicolosi and A. E. G. Cass, *Chem. Commun.*, 1997, 517.
15 R. Blonder, E. Katz, Y. Cohen, N. Itzhak, A. Riklin and I. Willner, *Anal. Chem.*, 1996, **68**, 3151.
16 B. F. Watkins, J. R. Behling, E. Kariv and L. L. Miller, *J. Am. Chem. Soc.* 1975, **97**, 3549; B. E. Firth and L. L. Miller, *J. Am. Chem. Soc.*, 1976, **98**, 8272.
17 V. I. Sokolov, L. L. Troitskaya and O. A. Rentov, *J. Organomet. Chem.* 1979, **182**, 537.
18 E. Laviron, *J. Electroanal. Chem.*, 1979, **101**. 19.
19 V. J. Razumas, A. V. Gudavicius and J. J. Kulys, *J. Electroanal. Chem.*, 1986, **198**, 81.

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