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[7] All new isolated compounds gave satisfactory analytical and/or spectral data (see Table 1).

[8] X-ray structural analyses: **1b**: Crystal size $0.38 \times 0.34 \times 0.20$ mm^3, space group $P2_1/c$ (no. 14), scan range $3 < 2\theta < 52.30°$, $a = 16.5909(3)$, $b = 9.1224(1)$, $c = 17.5914(2)$ Å, $\beta = 108.089(1)°$, $V = 2430.84(8)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.167$ g cm^{-3}, $\mu = 1.55$ cm^{-1}, 4743 unique reflections at −85 °C, of which 3320 were taken as observed $[F_o > 3.00\sigma(F)]$, $R = 0.036$, $R_w = 0.046$. **2b:** Crystal size $0.30 \times 0.25 \times 0.20$ mm^3, space group *Pbcn* (no. 60), scan range $4.00 > 2\theta > 45.00$, $a = 22.9475(3)$, $b = 12.8479(2)$, $c = 8.7785(2)$ Å, $V = 2588.14(7)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.351$ g cm^{-3}, $\mu = 0.77$ cm^{-1}, 2582 unique reflections at −101 °C, of which 1551 were taken as observed $[F_o > 3.00\sigma(F)]$, $R = 0.037$, $R_w = 0.042$. **2c**: Crystal size $0.25 \times 0.10 \times 0.10$ mm^3, space group $P2_12_12_1$ (no. 19), scan range $4.00 < 2\theta < 45.00°$, $a = 25.3045(3)$, $b = 5.3273(1)$, $c = 18.5416(4)$ Å, $V = 2499.49(7)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.219$ g cm^{-3}, $\mu = 0.69$ cm^{-1}, 4270 unique reflections at −108 °C, of which 2444 were taken as observed $[F_o > 3.00\sigma(F)]$, $R = 0.054$, $R_w = 0.065$. **8**: Crystal size $0.41 \times 0.24 \times 0.02$ mm^3, space group *Pbca* (no. 61), scan range $3.00 < 2\theta < 45.00°$, $a = 20.9160(5)$, $b = 7.5075(2)$, $c = 32.6655(7)$ Å, $V = 5129.35(29)$ Å^3, $Z = 8$, $\rho_{calcd} = 1.270$ g cm^{-3}, $\mu = 0.77$ cm^{-1}, 4210 unique reflections at −118 °C, of which 1873 were taken as observed $[F_o > 3.00\sigma(F)]$, $R = 0.029$, $R_w = 0.030$. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-103129 (**8**), 103130 (**2c**), 103131 (**2b**), 103132 (**1b**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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Coordinative Approach to Mediated Electron Transfer: Ruthenium Complexed to Native Glucose Oxidase**

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Mediated electron transfer to or from active sites of oxidoreductases[1] is often accomplished by covalent binding of a redox-active species either to the enzyme surface[2–5] or to the enzyme active site.[6] Transition metal complexes such as ferrocene derivatives and osmium compounds are most commonly used. In addition, there are reports on using ruthenium compounds;[7, 8] however, these did not find widespread application, and this seems rather surprising taking into account the lower cost of Ru compounds and their higher reactivity toward glucose oxidase (GO)[9] with respect to related Os complexes. Also, an attractive feature of ruthenium complexes of the type *cis*-$[Ru(LL)_2Cl_2]$ (LL = bpy or phen-type ligands) is associated with their ability to coordinate monodentate nitrogen donor ligands like pyridine or imidazole (Q) [Eq. (1)].

$$\textit{cis}\text{-}[\mathrm{Ru(LL)_2Cl_2}] + \mathrm{Q} \rightleftharpoons \textit{cis}\text{-}[\mathrm{Ru(LL)_2ClQ}]^+\mathrm{Cl}^- \tag{1}$$

If proteins are considered as potential ligands, enzymes appear to be superior, since many of them, including oxidoreductases, contain histidine residues in their active sites. Therefore, it was anticipated that the interaction between an enzyme and *cis*-$[Ru(LL)_2Cl_2]$ would result in binding of the ruthenium center to the imidazole side chain of histidine in such a way that it is delivered close to the active site and thus provides an efficient electron transfer relay (Scheme 1). Glucose oxidase from *A. niger* is a very advantageous enzyme because it contains two imidazole side chains, His516 and H559, close to FAD.[10] Therefore we decided to carry out a "coordinative" loading of the complexes *cis*-$[Ru(LL)_2Cl_2]$ (LL = bpy (**1**) and phen (**2**)) into GO and to investigate electrocatalytic characteristics of ruthenium-modified GO (Ru(LL)-GO). As a result, we succeeded in

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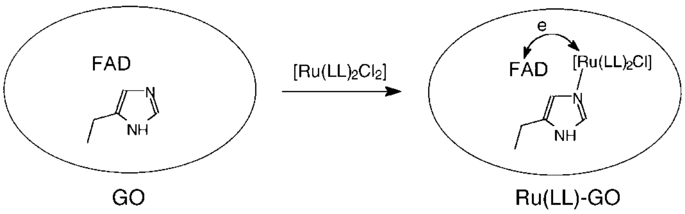
[**] Financial support from the Russian Foundation for Basic Research (Grant No 96-03-34328a), INTAS (Project 1432), and a fellowship from the Swedish Institute for ESR is gratefully acknowledged. We thank Den Bokhan for experimental assistance. Abbreviations: FAD: flavin adenine dinucleotide; GO: glucose oxidase.



Scheme 1. Schematic illustration of binding of a Ru complex with glucose oxidase and its relay function.

preparing an extremely active modified biocatalyst with superior electrocatalytic properties.

The redox potential of the $Ru^{III} + e \rightleftarrows Ru^{II}$ half-reaction is almost ideal for coupling with GO. Since Ru^{II} and Ru^{III} compounds have drastically different abilities to undergo ligand exchange,[11] the latter are practically inert to ligand substitution,[9] compounds of Ru^{II} should be used in the complex formation reaction with GO. After the complex formation, Ru^{II} should preferably be oxidized to Ru^{III} in order to keep the redox species more strongly bonded to the protein donor groups. A two-step procedure is utilized, which is reminiscent of development and fixation of black-and-white photographic films: complexation (development) and dialysis (fixation) were performed at different pH values.

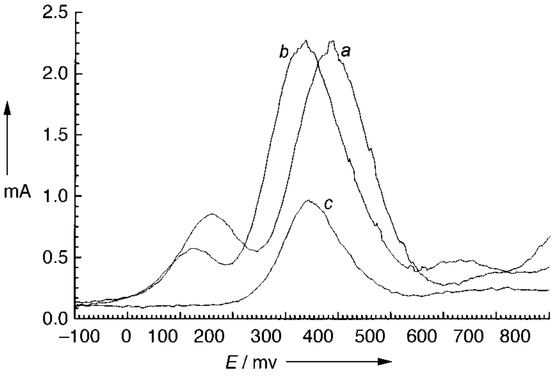


Figure 1. Differential pulse voltammograms of **2** (9.8×10^{-4} M; 0.01 M phosphate, pH 5.5, 25 °C, scan rate 20 mV s^{-1}) a) without addition of imidazole, b) in the presence of two equivalents of imidazole, and c) in the presence of 0.14 equivalents of GO.

Differential pulse voltammetry demonstrated that **2** interacts with imidazole in buffered aqueous solution at pH 5.5. Addition of two equivalents of imidazole to a solution of **2** results in a potential shift from 382 to 330 mV (Figure 1 a,b). Remarkably, a very similar shift, that is from 382 to 340 mV, is observed on addition of GO to the solution of **2** (Figure 1 c). The potential shift is accompanied by a 2.5-fold decrease in current which is in agreement with the estimated value of 2.3 made on assumption that the complex is completely bound to a macromolecule of 150000 Da.[12] The presence of one peak in Figure 1 c strongly suggests that Ru^{II} is completely bound to the enzyme and that the binding involves similar donor groups, very likely imidazole side chains of histidine residues. The same conclusions were reached in the case of complex **1**. It is known that Ru^{III} derivatives obtained, for instance, from **2** are readily reduced by D-glucose in the presence of GO, and that this interaction can be monitored by using UV/Vis spectroscopy.[9] The same behavior was confirmed by a cyclic voltammetry study. Both **1** and **2** display effective intermolecular coupling with GO, and the corresponding second-order rate constants *k* for the oxidation of reduced GO by an electrochemically generated Ru^{III} species are equal $(1.5 \pm 0.2) \times 10^{5}$[9] and $(0.90 \pm 0.06) \times 10^{5}\,M^{-1}s^{-1}$, respectively, (25 °C, pH 7 (0.01 M phosphate), [glucose] 0.033 M). Interestingly, at pH 7 (adjusted using 0.1 M phosphate) the rate constants are lower; in the case of **1** the rate constant *k* is $(5.4 \pm 0.2) \times 10^{4}\,M^{-1}s^{-1}$. However, under these conditions reaction (1) generates species *cis*-$[Ru(bpy)_2ClQ]^+$ (Q = py, imidazole or histidine) with increased formal redox potential which moves anodically by 182, 106, and 104 mV, respectively, relative to $E^{\circ\prime}$ of **1** (300 mV). This drastically increases *k* and the corresponding rate constants are $(4.15 \pm 0.01) \times 10^{5}$, $(3.6 \pm 0.2) \times 10^{5}$, and $(9.6 \pm 0.4) \times 10^{5}\,M^{-1}s^{-1}$, respectively. Rather surprisingly, the effect practically disappears in 0.01 M phosphate.

The complexation between **1** or **2** and GO was carried out in buffered phosphate solution (0.01 M) at pH 5.5; ratio of GO to Ru^{II} was 1:3.5 and 1:7, respectively. Under these conditions, Ru^{II} is not oxidized by dioxygen into Ru^{III}. Hence, the substitutionally labile species is involved in the "development" step. The dialysis is carried out against the same buffer of pH 7.0. At this pH value, slow oxidation into Ru^{III} is evident, and after 48 h practically all the ruthenium is converted into Ru^{III}. Thus, the ruthenium species becomes fixed to the donor groups of the enzyme. In the case of the above indicated GO to Ru^{II} ratios, all ruthenium binds to the enzyme. It was verified that the properties of the Ru-enriched enzyme do not change after gel filtration, and it is the dialysis coupled with the oxidation that is required for preparation of the active biocatalyst.

The Ru-modified GO (Ru(LL)-GO) was shown to preserve both high catalytic activity and stability. A commonly observed range of the catalytic activity measured spectrophotometrically with respect to 2,6-dichlorophenolindophenol (DCIP)[13] is 73–82 % as compared to native GO. The stability of Ru(LL)-GO is significant and any loss in activity was not observed after keeping a solution of Ru((LL)-GO for one month at pH 7 and 5 °C. Its functional stability is indistinguishable from that of native GO. UV/Vis spectra of Ru(LL)-GO are a superposition of that of GO and Ru^{III} (Figure 2). In particular, Ru(phen)-GO has maxima at 455 and 648 nm arising from FAD and Ru^{III}, respectively (Figure 2 a). Addition of D-glucose induces a color change of Ru(phen)-GO from greenish to purple (Figure 2 b), which is attributed to conversion of the coordinated Ru^{III} into Ru^{II} [Eq. (2); GO(ox) and GO(red) are the oxidized and reduced forms of the enzyme, respectively]. As a result, the spectrum of

$$Ru^{III}\text{-GO(ox)} \xrightarrow{\text{D-glucose}} Ru^{II}\text{-GO(red)} \qquad (2)$$

reduced Ru(phen)-GO in the range 400–800 nm is similar to that of **2** recorded in the presence of imidazole (Figure 2 c). In particular, the band at 648 nm disappears and the intensity of the 455 nm band drops since FAD is reduced to $FADH_2$.[14] There is a shift in the absorption maximum to 467 nm which

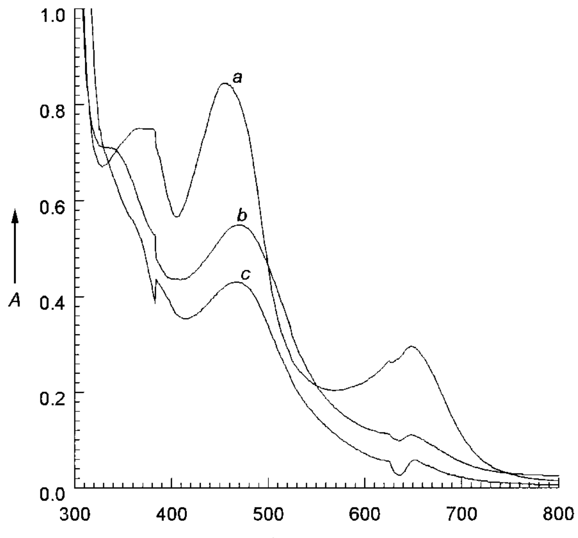


Figure 2. UV/Vis spectra of Ru(phen)-GO (3.4 mg mL^{-1}) a) before and b) after reduction by excess of D-glucose (0.1 M). c) UV/Vis spectrum of **2** in the presence of imidazole. Conditions for all spectra: pH 7 (0.01 M phosphate), 22 ± 2 °C.

corresponds to Ru^{II}. Since the enzymatic activity is about 75 % and assuming that Ru^{II} is the only absorbing species at this wavelength, the amount of the "catalytically active" ruthenium can be estimated by using the extinction coefficient obtained for **2** in the presence of imidazole. Calculations suggest that Ru(phen)-GO and Ru(bpy)-GO have three and one active ruthenium centers, respectively.

The electrocatalytic properties of the Ru(LL)-GO preparations as demonstrated by cyclic voltammetry are presented in Figure 3. There are only weak signals from ruthenium coordinated to glucose oxidase in the absence of D-glucose at 350 and 324 mV for Ru(phen)-GO and Ru(bpy)-GO, respectively, at a scan rate of 10 mV s^{-1} (traces a). Traces b in Figure 3 recorded in the presence of D-glucose emphasize the impressive electrocatalytic properties of Ru(LL)-GO. In fact, the amplification coefficient I/I_o is 12.8 for Ru(bpy)-GO and 66.7 for Ru(phen)-GO, (at 500 and 480 mV, respectively, and 10 mV s^{-1} scan rate). The amplification coefficients obtained for both preparations are summarized in Table 1. Remarkably, these are significant even at high scan rates and this makes the ruthenium-modified catalysts very promising for biosensor applications.

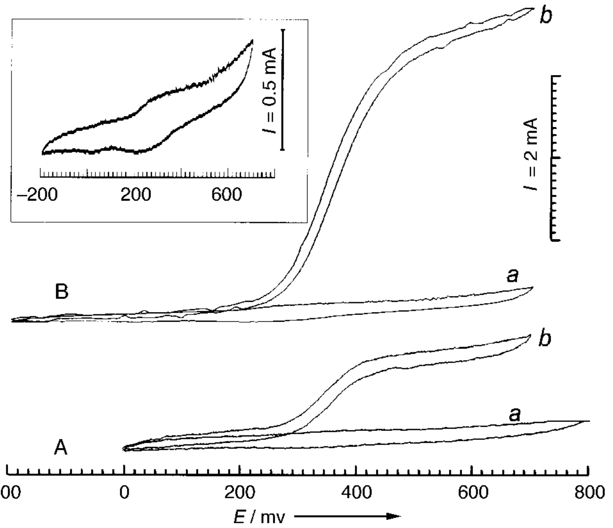


Figure 3. Cyclic voltammograms of Ru(bpy)-GO (A) and Ru(phen)-GO (B) a) in the absence and b) in the presence of D-glucose (0.033 M). [Ru(bpy)-GO] 12.7, [Ru(phen)-GO] 17.1 mg mL^{-1}, pH 7 (0.01 M phosphate buffer), scan rate 10 mV s^{-1}. The inset shows magnified voltammogram of Ru(phen)-GO in the absence of D-glucose.

Table 1. Amplification coefficients I/I_o at 500 (Ru(bpy)-GO) and 480 mV (Ru(phen)-GO) obtained at different scan rates v; 25 °C, pH 7 (0.01 M phosphate).

	v [mV s^{-1}]	I/I_o		v [mV s^{-1}]	I/I_o
Ru(bpy)-GO	2	40.9	Ru(phen)-GO	5	93.7
	5	37.8		10	66.7
	10	12.8		20	47.6
				30	20.5
				40	18
				100	7.1

In order to compare the efficacy of Ru(LL)-GO with the enzyme modified by other redox species, the rate constants for intramolecular electron transfer were estimated by computer simulation of the data in Figure 3 by known methods.[15]

We applied the model summarized in Scheme 2 and obtained the rate constants 70 s^{-1} for Ru(phen)-GO and 12 s^{-1} for Ru(bpy)-GO. For comparison the highest rate constant reported for GO randomly modified with 13 ferrocenecarboxylic acid residues is 3.6 s^{-1}.[16] We also showed that random modification of GO with 4-pyridylacetic acid followed complexation with **1** resulted in the catalyst with $k_1 = 0.55$ s^{-1}. Advantages of the coordinative modification require no further comment.

$$\mathrm{FADH_2\text{-}M^{II}} \rightleftharpoons \mathrm{FADH_2\text{-}M^{III}} + \mathrm{e}\ (E_1^{0'},\ \text{electrode})$$

$$\mathrm{FADH_2\text{-}M^{III}} \longrightarrow \mathrm{FADH^{\bullet}\text{-}M^{II}} + \mathrm{H^+}\ (k_1)$$

$$\mathrm{FADH^{\bullet}\text{-}M^{II}} \rightleftharpoons \mathrm{FADH^{\bullet}\text{-}M^{III}} + \mathrm{e}\ (E_2^{0'},\ \text{electrode})$$

$$\mathrm{FADH^{\bullet}\text{-}M^{III}} \longrightarrow \mathrm{FAD\text{-}M^{II}} + \mathrm{H^+}\ (k_2)$$

$$\mathrm{FAD\text{-}M^{II}} \longrightarrow \mathrm{FADH_2\text{-}M^{II}}\ (k_3)$$

assumptions: $k_1 = k_2$, $E_1^{0'} = E_2^{0'}$,[16] $k_3 = 800$ s^{-1} (saturating glucose concentration)[14]

Scheme 2. The model applied for the computer simulation.

In conclusion, the extremely simple coordinative modification of GO with Ru^{II}(bpy) or Ru^{II}(phen) complexes affords very catalytically and electrocatalytically active, stable biocatalysts which provide an unprecedently high amplification coefficient I/I_o even at high scan rates and, correspondingly, very high rates of intramolecular electron transfer. This strongly suggests that the ruthenium centers are located close to the enzyme active site and coordinated presumably to 516 and/or 559 histidines.

Experimental Section

Electrochemical measurements were carried out using a potentiostat/galvanostat Model 273A (EG&G Prinston Applied Research). A three-electrode scheme was used with a working glassy carbon electrode, saturated AgCl/Ag reference electrode, and an auxiliary Pt electrode. GO from *Aspergillus niger* (EC1.1.3.4, 285 $\mathrm{U\,mg^{-1}}$) was a Serva preparation. Ruthenium(II) complexes were prepared as described.[17]

Modification of GO by $\mathrm{Ru^{II}}$: To a solution of **2** (1 mL, 9.8×10^{-4} M) in 0.01M phosphate buffer (pH 5.5) a weighed amount of GO (20.5 mg) was added (Ru:GO ratio 7:1) to yield a brownish red solution. The mixture was kept at room temperature for 4 h during which the solution faded, which was attributed to binding to GO and partial oxidation of $\mathrm{Ru^{II}}$ to $\mathrm{Ru^{III}}$. The solution was then dialyzed against 0.01M phosphate buffer (1 L, pH 7.0) for 48 h at 5 °C, and the buffer was changed at least twice. A green solution was finally formed indicative of complete oxidation of $\mathrm{Ru^{II}}$ to $\mathrm{Ru^{III}}$. Analysis of the dialyzed solution by differential pulse voltammetry indicated the absence of free ruthenium complex not bound to GO . The amount of protein in the sample as determined according to the Lowry method was 17.1 $\mathrm{mg\,mL^{-1}}$; the activity determined spectrophotometrically with respect to DCIP was 73.4% relative to the native GO with 100% activity. Modification of GO by **1** was carried out in a similar way by using a Ru to GO ratio of 3.5:1. The activity was 82%.

Received: July 1, 1998 [Z12079IE]
German version: *Angew. Chem.* **1999**, *111*, 847 – 850

Keywords: electrocatalysis · electron transfer · glucose oxidase · ruthenium

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Neutral, Cationic, and Anionic Alkyl Derivatives of Tungsten Bonded to a Calix[4]arene Oxo Surface**

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Particular attention is focused on those systems which can mimic at the molecular level the chemical behavior of M–C functionalities bonded to an oxo surface.[1] The most common approach employs alkoxo and phenoxo groups as ancillary ligands.[2] However, a quite recent and significant advance in the field was achieved with the preorganized set of oxygen donor atoms from calix[4]arenes,[3] which bear a number of unique peculiarities[4] within this context. We report here the generation of a dimethyl tungsten(VI) derivative bonded to an $\mathrm{O_4}$ set of donor atoms, its conversion into an unsaturated monomethyl cation, and its very peculiar redox behavior.

The alkylation of *cis*-$[(\mathrm{Cl})_2\mathrm{W}\{p\text{-}t\mathrm{Bu\text{-}calix[4]\text{-}(O)_4}\}]$[5] (**1**) is strongly dependent on the alkylating agent. The use of carbanionic, reducing species, like lithium and magnesium organometallic compounds, led straight to the corresponding anionic alkylidynes,[4b] while the use of $\mathrm{ZnMe_2}$ in toluene in a 1:1 ratio led cleanly to *cis*-$[(\mathrm{Me})_2\mathrm{W}\{p\text{-}t\mathrm{Bu\text{-}calix[4]\text{-}(O)_4}\}]$ (**2**, Scheme 1). The use of smaller amounts of $\mathrm{ZnMe_2}$ produced mixtures of **1** and **2**. The monoalkyl derivative $[(\mathrm{Cl})(\mathrm{Me})\mathrm{W}\{p\text{-}t\mathrm{Bu\text{-}calix[4]\text{-}(O)_4}\}]$ (**3**) could be best prepared by photolysis of **1** in $\mathrm{CH_2Cl_2}$ in the presence of an excess of pyridinium hydrochloride (PyHCl). As **1** does not react with PyHCl in the dark and the photolysis of **1** in $\mathrm{C_6D_6}$ led to evolution of methane and ethylene (^{1}H NMR), we believe that PyHCl traps an unstable methylene species generated by photolysis.[6, 7] Compound **3** was also obtained by a ligand exchange reaction of **2** with $\mathrm{SnCl_4}$.

The apparent $C_{4\mathrm{v}}$ symmetry of the calix[4]arene fragment, as determined from the ^{1}H NMR spectra at 298 and 193 K, is a consequence of the equal availability of d_{xz} and d_{yz} as well as d_{z^2} orbitals for binding of the two methyl groups. This result emphasizes the very high mobility of the alkyl groups between two equivalent positions over the oxo surface and the absence of a significant energy barrier for movement of the methyl groups between them. In the case of **3**, the $C_{4\mathrm{v}}$ symmetry at 298 K is modified to C_{s} in the ^{1}H NMR spectrum measured at 188 K. The difference in the metal-to-ligand bonding mode for Me (σ only) and Cl substituents (σ and π) is a factor in the increased barrier to mobility of the ligands Me and Cl over the oxo surface.

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[**] This work was supported by the Fonds National Suisse de la Recherche Scientifique (Bern, Switzerland, grant no. 20-53336.98) and Ciba Specialty Chemicals (Basel, Switzerland).