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Dipolar ruthenium(II) ammine complexes as electron transfer mediators of amperometric glucose sensors

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Abstract

The mediation of dipolar ruthenium(II) ammine complexes containing pyridinium ions $[Ru(NH_3)_5(L^+)]^{3+}$(L^+: pyridinium ions) in glucose oxidation has been investigated by a voltammetric method. These ruthenium(II) complexes had appropriate redox potentials of 0.10–0.18 V vs. Ag/AgCl and high k_s values of $5.7–17 \times 10^6$ M^{-1} s^{-1} which are the second-order rate constants for electron transfer from glucose oxidase in reduced form to $[Ru(NH_3)_5(L^+)]^{4+}$. In particular, the k_s values for $[Ru(NH_3)_5(L^+)]^{3+}$ were greater than those of osmium(II)–polypyridine complexes possessing similar redox potentials which are most commonly used. All the dipolar ruthenium(II) complexes used in this study are therefore concluded to be useful for the electron transfer mediators of amperometric glucose sensors.

Keywords: Ruthenium(II) complexes; Mediator; Glucose sensor; Electrocatalysis

1. Introduction

The enzyme glucose oxidase (GOx, EC 1.1.3.4) specifically converts glucose into gluconolactone, which subsequently hydrolyzes spontaneously to gluconic acid with concomitant reduction of dioxygen to hydrogen peroxide. This oxidation reaction is accompanied by reduction of the cofactor flavin adenine dinucleotide (FAD). The flavin cofactor FAD is responsible for the redox properties of the enzyme, and available evidence suggests that it is firmly bound, but not covalently linked, to the polypeptide protein of the enzyme. This enzyme GOx, a structurally rigid glycoprotein, is most widely used as a biosensor in an increasing number of clinical, environmental, agricultural, and biotechnological applications. Thus, GOx accepts electrons from glucose through the flavin moiety and transfers them to dioxygen but not to simple electrodes. The reason that reduced flavin adenine dinucleotide ($FADH_2$) known as the active site is not oxidized electrochemically through the electrode reaction:

$$FADH_2 \rightarrow FAD + 2H^+ + 2e^- \qquad (1)$$

is that the FAD/$FADH_2$ redox centers of the homodimer are located the depth of GOx from *Aspergillus niger* active site pocket of ca. 10 Å [1]. So the idea is developed to use a non-physiological redox-active molecule as an electron acceptor for GOx in reduced form, which is usually called a mediator. Numerous artificial electron acceptors are used as electron transfer mediators for coupling the glucose/GOx system to an electrode surface. Transition metal complexes such as iron [2–4] and osmium [5–15] compounds are most commonly used. In addition, there are papers on using ruthenium complexes [5,6,12,16–20]. In a recent work, Dempsey et al. have evaluated novel mediators for a glucose biosensor based on ruthenium and osmium picolinate complexes [21]. Ruthenium complexes are of greater advantage than related osmium complexes owing to the lower cost and their higher reactivity toward GOx [12]. Taking the example of glucose, one can be describe the operation of a ruthenium complex as an electron transfer mediator as follows:

$$\beta-\mathrm{D}-\text{glucose} + \mathrm{GOx_{ox}(FAD)} \rightarrow \text{glucono}-\delta - \text{lactone} + \mathrm{GOx_{red}(FADH_2)} \qquad (2)$$

$$\mathrm{GOx_{red}(FADH_2) + 2Ru(III) \rightarrow GOx_{ox}(FAD) + 2Ru(II)} \qquad (3)$$

$$\mathrm{2Ru(II) \rightarrow 2Ru(III) + 2e^-} \qquad (4)$$

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where $GOx_{ox}(FAD)$ and $GOx_{red}(FADH_2)$ are oxidized and reduced forms of GOx, respectively. A ruthenium complex as an electron transfer mediator is required to have (1) an appropriate redox potential of ruthenium(III/II) couple that in turn enables the electrode to be polarized at a potential that does not give rise to interfering electrochemical reaction, (2) chemical stability in both oxidized and reduced forms, and (3) a high value of k_s, which is the second-order rate constant for electron transfer from $GOx_{red}(FADH_2)$ shown as Eq. (3), to minimize competition with dioxygen. Consequently, the choice of the electron transfer mediator is certainly important for achieving high sensitivity and selectivity of amperometric glucose sensors.

The reactivity between a metal complex and GOx_{red} $(FADH_2)$ may be discussed through the use of Marcus relationship [22,23] for the electron transfer reaction:

$$k_s = (k_{11}k_{22} f K_s)^{1/2} \qquad (5)$$

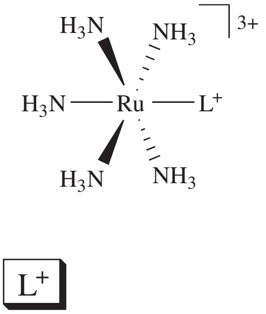
where k_s is the second-order rate constant for the electron transfer cross-reaction between the mediator and GOx (Eq. (3)), k_{11} and k_{22} are the self-exchange rate constants for the mediator and GOx, K_s is the equilibrium constant of Eq. (3). Frequently, f is close to unity. Differential excitation flash photolysis work has shown that the k_{11} values of $[M(PP)_3]^{3+/2+}$ complexes (M=Fe, Ru, PP=polypyridine) are in the range of 10^8–10^9 M^{-1} s^{-1} [24], and 10^3–10^5 times those which have been obtained for $[Ru(NH_3)_6]^{3+}$ (2.5×10^5 M^{-1} s^{-1}) and $[Ru(NH_3)_5(py)]^{2+}$ (py=pyridine, 1.7×10^4 M^{-1} s^{-1}) [23]. However, $[Ru(PP)_3]^{3+/2+}$ (PP=2,2′-bipyridine, 1,10-phenanthroline) complexes can not act as the electron transfer mediators for glucose oxidation since the redox potentials of Ru(III/II) couples are too high (>1.0 V vs. Ag/AgCl). On the other hand, $[Ru(NH_3)_5(py)]^{2+}$ complex has possessed the small k_s value (1×10^5 M^{-1} s^{-1}) although the redox potential of Ru(III/II) couple is satisfactorily low ($E_{1/2}$=0.09 V vs. Ag/AgCl) [25].

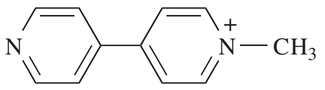
In this paper, we describe the results of studies on the interactions between GOx and dipolar ruthenium(II) complexes $[Ru(NH_3)_5(L^+)]^{3+}$ as shown in Fig. 1, which are chemically stable in both oxidized and reduced forms because of the electron-withdrawing effect of the pyridinium ions L^+. The factors to be satisfied by electron transfer mediators for amperometric glucose sensors are discussed, and the evaluation of $[Ru(NH_3)_5(L^+)]^{3+}$ as electron transfer mediators is made on the basis of the redox potentials of ruthenium(III/II) couples and the second-order rate constants for electron transfer between $GOx_{red}(FADH_2)$ and the ruthenium(III) complex.

2. Experimental

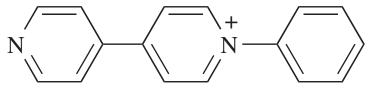
2.1. Materials

The complex $[RuCl(NH_3)_5]Cl_2$, ammonium hexafluorophosphate, and D-glucose were supplied by Wako Pure Chemicals. GOx from *A. niger* (EC 1.1.3.4, 25 U/mg of solid, type II, molecular weight 1.86×10^5) [26] was purchased from Sigma. All other chemicals were of reagent grade and were used as received. A stock aqueous solution of glucose (1 M (M=mol dm^{-3})) was left for at least 24 h at room temperature to allow for equilibration of the anomers. All aqueous solutions were prepared with Millipore “Milli Q” grade water.

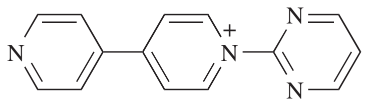




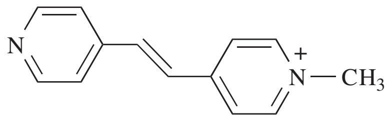
N-methyl-4,4′-bipyridinium (MeQ+)



N-phenyl-4,4′-bipyridinium (PhQ+)



N-(2-pyrimidyl)-4,4′-bipyridinium (PymQ+)



N-methyl-4-[*trans*-2-(4-pyridyl)ethenyl]pyridinium (Mebpe+)

Fig. 1. Structures of ruthenium(II) complex and ligands used in this study.

2.2. Physical measurements

Cyclic voltammograms were obtained by using an Als/chi electrochemical analyzer model 600A with a 20-mL one-compartment three-electrode electrochemical cell. A three-electrode system was used with a 3-mm diameter glassy carbon working electrode (BAS 11-2012), an Ag/AgCl reference electrode (BAS 11-2020), and a platinum wire counter electrode. Cyclic voltammetric measurements were carried out in deaerated 0.1 M phosphate buffer (pH 7.0) at 25.0±0.1 °C. Absorption spectra were recorded on a Shimadzu UV-3101PC (quartz cell length was 10 mm).

2.3. Syntheses

The ligands *N*-methyl-4,4′-bipyridinium iodide ($[MeQ^+]I$) [27], *N*-phenyl-4,4′-bipyridinium chloride ($[PhQ^+]Cl$) [28], *N*-

(2-pyrimidyl)-4,4′-bipyridinium chloride ([PymQ$^+$]Cl) [29], and *N*-methyl-4-[*trans*-2-(4-pyridyl)ethenyl]pyridinium iodide ([Mebpe$^+$]I) [29] were prepared according to published procedures. The complexes [Ru(NH$_3$)$_5$(L$^+$)]Cl$_3$ (L$^+$ = MeQ$^+$, PhQ$^+$, PymQ$^+$, Mebpe$^+$) were synthesized from [RuCl(NH$_3$)$_5$]Cl$_2$ via slight modifications of literature methods [28,30]. Absorption spectral data of [Ru(NH$_3$)$_5$(L$^+$)]Cl$_3$ in CH$_3$CN were as follows. [Ru(NH$_3$)$_5$(MeQ$^+$)]Cl$_3$: λ_{max}/nm (ε_{max}/M^{-1} cm^{-1}) = 587 (1.53×10^4), 267 (1.65×10^4) [lit.: 590 (1.58×10^4), 268 (1.63×10^4)] [28]. [Ru(NH$_3$)$_5$(PhQ$^+$)]Cl$_3$: λ_{max}/nm (ε_{max}/M^{-1} cm^{-1}) = 625 (2.04×10^4), 280 (1.93×10^4) [lit.: 628 (1.93×10^4), 280 (1.85×10^4)] [28]. [Ru(NH$_3$)$_5$(PymQ$^+$)]Cl$_3$: λ_{max}/nm (ε_{max}/M^{-1} cm^{-1}) = 677 (1.76×10^4), 284 (2.06×10^4) [lit.: 673 (1.80×10^4), 285 (2.15×10^4)] [29]. [Ru(NH$_3$)$_5$(Mebpe$^+$)]Cl$_3$: λ_{max}/nm (ε_{max}/M^{-1} cm^{-1}) = 595 (1.62×10^4), 312 (2.40×10^4) [lit.: 595 (1.61×10^4), 312 (2.38×10^4)] [29].

2.4. Kinetic measurements

The second-order rate constant for a given electron transfer mediator was determined by cyclic voltammetry according to the experimental method described by Cass et al. [31] and used frequently in the literature. The magnitude of the catalytic current at a glassy carbon electrode was measured in samples with increasing concentrations of GOx$_{ox}$ (2–12 μM) containing 0.1 M glucose and 0.1 mM ruthenium(II) complex at the scan rate of 1 to 20 mV s^{-1}. The change in the catalytic current with the addition of GOx$_{ox}$ was compared with the diffusion current obtained in the absence of GOx$_{ox}$ at various scan rates and thereby related to the rate constant by the scheme of Nicholson and Shain [32].

3. Results and discussion

3.1. Characterization of [Ru(NH$_3$)$_5$(L$^+$)]$^{3+}$

Absorption spectral and electrochemical data of [Ru(NH$_3$)$_5$(L$^+$)]$^{3+}$ in 0.1 M phosphate buffer (pH 7.0) are shown in Table 1. All the ruthenium(II) complexes show intense and broad d$_\pi$(Ru^{II}) → π*(L$^+$) metal-to-ligand charge-transfer (MLCT) bands in the region 580–677 nm, the energies of which are related to the electron donor ability of the ruthenium center and to the electron-acceptor ability of L$^+$ [33,34]. The MLCT band maximum of PymQ$^+$ complex is red-shifted by ca. 100 nm with respect to MeQ$^+$ complex. Given an almost constant HOMO energy, this shows that PymQ$^+$ has the lowest LUMO energy of the 4,4′-bipyridinium ligands used in this study, in keeping with the highly electron-deficient nature of the 2-pyrimidyl group. The acceptor strength of L$^+$ hence increases in the order MeQ$^+$ < PhQ$^+$ < PymQ$^+$. The MLCT energy of Mebpe$^+$ complex is very similar to that of MeQ$^+$ complex.

Table 1
Absorption spectral data of [Ru(NH$_3$)$_5$(L$^+$)]Cl$_3$ and redox potentials of Ru(III/II) couples in 0.1 M phosphate buffer (pH 7.0)

L$^+$	λ_{max} / nm (ε_{max} / M^{-1} cm^{-1})	Assignment	$E_{1/2}$^{a} / V vs. Ag/AgCl (ΔE_p / mV)
MeQ$^+$	580 (11 300)	d$_\pi$ → π*(MeQ$^+$)	0.152 (60)
PhQ$^+$	617 (20 400)	d$_\pi$ → π*(PhQ$^+$)	0.165 (60)
PymQ$^+$	677 (14 800)	d$_\pi$ → π*(PymQ$^+$)	0.180 (63)
Mebpe$^+$	588 (10 100)	d$_\pi$ → π*(Mebpe$^+$)	0.101 (60)

[a] Scan rate = 50 mV s^{-1}.

As shown in Table 1, all the ruthenium(II) complexes exhibit reversible ruthenium(III/II) waves and possess the redox potentials of ruthenium(III/II) couples in the region 0.10–0.18 V vs. Ag/AgCl. In the 4,4′-bipyridinium ligands, the redox potentials increase in the order MeQ$^+$ < PhQ$^+$ < PymQ$^+$. This order is in agreement with the acceptor strength of L$^+$, that is, the stronger the electron-acceptor ability of L$^+$, the more positive the redox potential of ruthenium(III/II) couple. However, the variations in the redox potentials are only small, showing that the HOMO energy is relatively insensitive to changes in the substituent at nitrogen. The corresponding red (bathochromic) shifts in the MLCT bands are mainly a result of stabilization of the ligand-based LUMOs. The redox potential of Mebpe$^+$ complex is shifted by ca. −50 mV with respect to that of MeQ$^+$ complex, due to the mildly electron-donating character of the ethylene unit.

3.2. Mediation of ruthenium complexes in glucose oxidation

The function of the ruthenium complexes as electron transfer mediators can be described according to the reaction mechanisms as shown in Eqs. (2)–(4). The two important properties for an electron transfer mediator are particularly required to fabricate the amperometric glucose sensors. First, an electron transfer mediator should possess an appropriate redox potential for the electrode reaction given by Eq. (4). Amperometric glucose sensors are necessary to detect glucose at near 0.0 V vs. Ag/AgCl, where the risk for interfering reactions is minimized and also where the background current and noise levels are the lowest. Second, in order to enhance the sensitivity and minimize competition with dissolved dioxygen, an electron transfer mediator should possess a high value of second-order rate constant k_s in Eq. (3).

The features of the cyclic voltammograms for all the ruthenium(II) complexes were attributed to the ruthenium(III/II) couples and to reversible electron transfer, with the anodic and cathodic peak heights being proportional to the square root of the scan rate. Table 1 summarizes the half-wave potentials ($E_{1/2}$) of the ruthenium(III/II) couples corresponding to the redox potentials and the peak separations (ΔE_p) between the two peak potentials. The ΔE_p values of 60–63 mV indicate that the electron transfers of all the ruthenium(III/II) couples are electrochemically reversible, i.e., the rates of heterogeneous electron transfer are fast. As the redox potentials of all the ruthenium(III/II) complexes were more positive than the redox potential of the FAD/FADH$_2$ couple (−0.41 V vs. Ag/AgCl at pH 7.0) [35], the mediation reaction as shown in Eq. (3) is considered thermodynamically possible. As previously described, amperometric glucose sensors should preferably detect glucose at near 0.0 V. Although [Ru(NH$_3$)$_5$(Mebpe$^+$)]$^{3+}$ may be considered as the best electron transfer mediator in all the ruthenium(II) complexes as evaluated solely by the redox

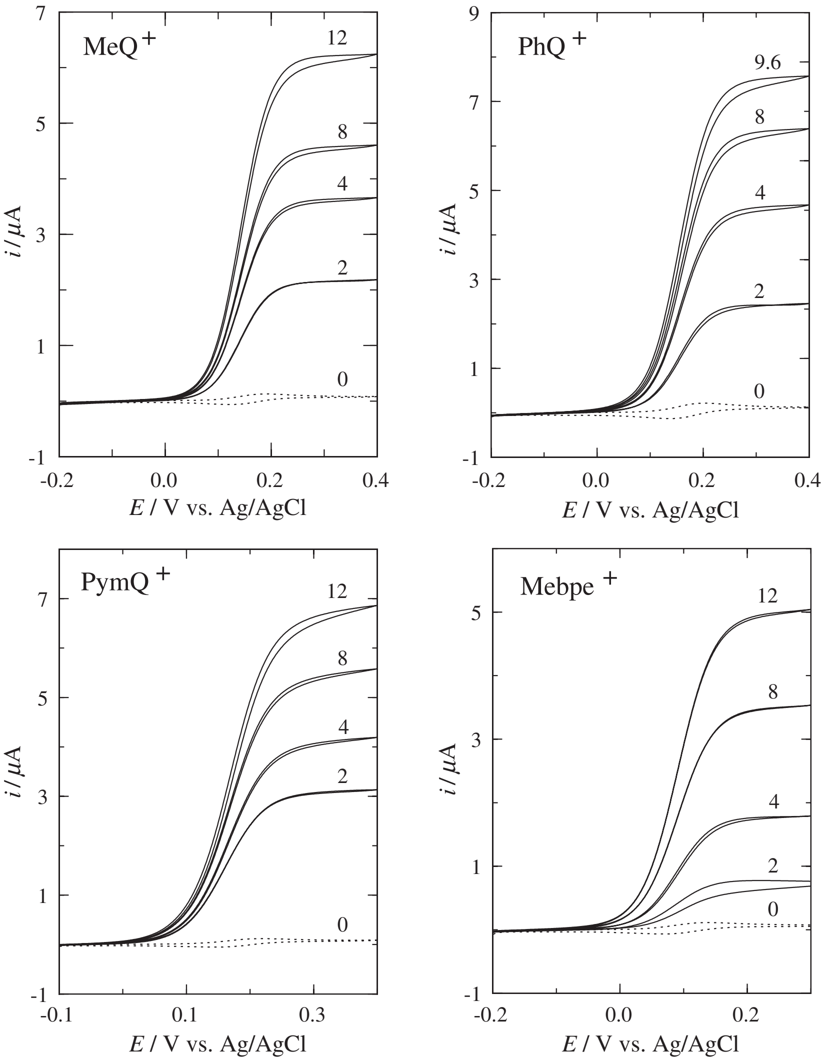


Fig. 2. Cyclic voltammograms of 0.1 mM $[Ru(NH_3)_5(L^+)]^{3+}$ in 0.1 M phosphate buffer (pH 7.0) containing 0.1 M glucose in the absence (dotted lines) and presence of GOx_{ox} (solid lines, μM). Scan rate = 2 mV s^{-1}.

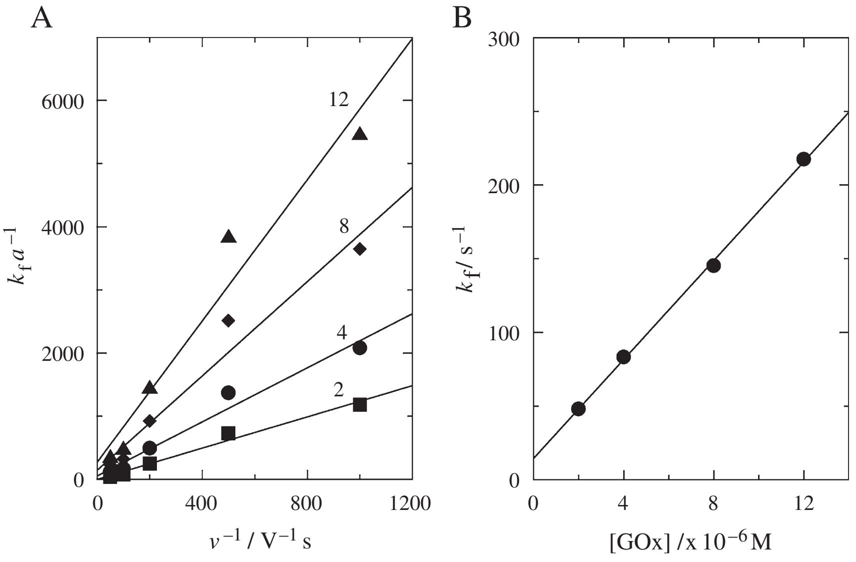


Fig. 3. (A) Plots of the kinetic parameter $k_f a^{-1}$ as a function of v^{-1} for various GOx_{ox} concentrations (μM) for $[Ru(NH_3)_5(PymQ^+)]^{3+}$. (B) Plot of the pseudo first-order rate constant k_f as a function of GOx_{ox} concentrations for $[Ru(NH_3)_5(PymQ^+)]^{3+}$.

Table 2
Redox potentials of Ru(III/II) couples and rate constants of glucose oxidation at pH 7.0 and 25 °C

Complex	$E_{1/2}$ / V vs. Ag/AgCl (ΔE_p / mV)	k_s / M^{-1} s^{-1}
$[Ru(NH_3)_5(MeQ^+)]^{3+}$	0.152 (60)	9.11×10^6
$[Ru(NH_3)_5(PhQ^+)]^{3+}$	0.165 (60)	5.68×10^6
$[Ru(NH_3)_5(PymQ^+)]^{3+}$	0.180 (63)	1.68×10^7
$[Ru(NH_3)_5(Mebpe^+)]^{3+}$	0.101 (60)	8.01×10^6
$[Os(Him)_2(dmbpy)_2]^{2+}$ [a]	0.154 (60)	2.26×10^6
$[OsCl(Him)(bpy)_2]^{+}$ [a]	0.107 (59)	1.21×10^5

[a] Him: imidazole, dmbpy: 4,4′-dimethyl-2,2′-bipyridine, bpy: 2,2′-bipyridine [14].

potentials of the ruthenium(III/II) complexes, the second-order rate constant k_s for the electron transfer reaction (Eq. (3)) must also be considered for the evaluation of electron transfer mediators.

The second-order rate constant for the reaction as shown in Eq. (3) can be estimated by the electrochemical method [31]. When both glucose and GOx_{ox} were added together to a solution of a ruthenium(II) complex, so-called catalytic current waves were observed for all the ruthenium(II) complexes (see Fig. 2). The magnitude of the catalytic current at the glassy carbon electrode was measured for sample solutions containing increasing amounts of GOx_{ox} with the bulk concentrations of ruthenium(II) complex and glucose kept constant. The change in the catalytic current i_k with additions of GOx_{ox} was compared with the diffusion current i_d obtained in the absence of GOx_{ox}. Using a working curve (see Fig. 14 of Nicholson and Shain [32]) of $i_k i_d^{-1}$ vs. kinetic parameter, $(k_f a^{-1})^{1/2}$, we replotted the data as $k_f a^{-1}$, where k_f is the pseudo first-order rate constant for mediation of glucose oxidation and $a = FvR^{-1}T^{-1}$ (F: Faraday constant, v: scan rate, R: gas constant, T: absolute temperature), against v^{-1} to obtain straight lines under first-order conditions, as shown in Fig. 3A for $[Ru(NH_3)_5(PymQ^+)]^{3+}$ at various GOx_{ox} concentrations. From the slope of each line, the corresponding scan rate-independent k_f value was obtained. The second-order rate constant k_s in Eq. (3) was next determined from the slope of the plot of the k_f value against the concentration of GOx_{ox} (Fig. 3B). Table 2 summarizes the k_s values determined for all the ruthenium(II) complexes and two osmium(II)–polypyridine complexes possessing similar redox potentials [14,15]. The k_s values of $5.7\text{–}17\times10^6$ M^{-1} s^{-1} for $[Ru(NH_3)_5(L^+)]^{3+}$ are greater than those of osmium(II) complexes. In particular, the rate constant for $[Ru(NH_3)_5(PymQ^+)]^{3+}$ with monodentate $PymQ^+$, of 1.68×10^7 M^{-1} s^{-1}, is very close to the upper limit for the best organic [36] and inorganic [19,37] mediators of GOx.

Ferrocene and its derivatives have been most extensively characterized and satisfied most of the criteria mentioned previously. However, the oxidized forms, the ferricinium cations, undergo slow hydrolysis in aqueous solution [38], and ferrocene and its derivatives are not readily soluble in aqueous solution, which may cause problems in the fabrication of glucose sensors. On the other hand, both the ruthenium (II) and ruthenium(III) complexes used in this study were stable and easily soluble in aqueous solution. In addition, the k_s value of 2.6×10^4 M^{-1} s^{-1} for ferrocene [31] is significantly smaller than that of a dipolar ruthenium(II) complex. These facts suggest that the electrostatic ruthenium(III/II) complex–GOx interaction and/or "antenna effect" of pyridinium ions introduced into GOx may contribute to greater k_s values.

For a glucose sensor to be of practical use, it is proposed that the enzyme GOx and an electron transfer mediator $[Ru(NH_3)_5(L^+)]^{3+}$ complex can be immobilized in electropolymerized films [39] or onto the surface of an electrode by polyion complex formation [40,41]. In particular, the surface functionalization of an electrically conductive polymer film (for example, polypyrrole film) with a bipyridinium ion may be effective for the covalent immobilization of GOx [42]; that is, $[Ru(NH_3)_5(L^+)]^{3+}$ complexes lead to electrical wiring of GOx.

3.3. Interactions of GOx with $[Ru(NH_3)_5(L^+)]^{3+}$

The k_s values for the dipolar ruthenium(II) complexes bearing a net charge of 3+ were greater than the values for the osmium(II) complexes bearing net charges of 1+ and 2+,

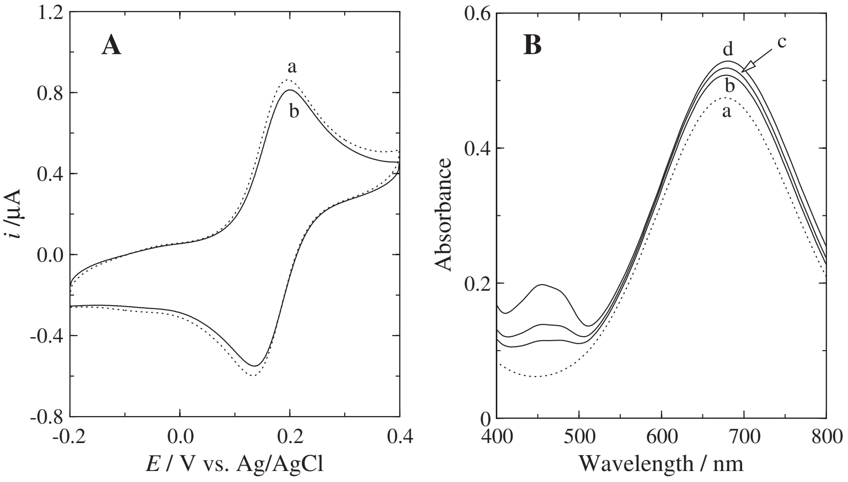
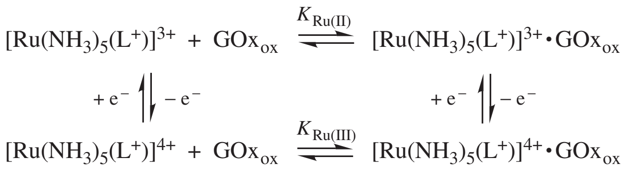


Fig. 4. (A) Cyclic voltammograms of 0.2 mM $[Ru(NH_3)_5(PymQ^+)]^{3+}$ in 0.1 M phosphate buffer (pH 7.0) in the absence (a) and presence (b) of 0.1 mM GOx_{ox}. Scan rate = 50 mV s^{-1}. (B) Absorption spectra of 50 μM $[Ru(NH_3)_5(PymQ^+)]^{3+}$ in 0.1 M phosphate buffer (pH 7.0) in the absence (a) and presence [(b) 20, (c) 30, (d) 50 μM] of GOx_{ox}.



Scheme 1. Adducts formation between $[Ru(NH_3)_5(L^+)]^{4+/3+}$ and GOx_{ox}.

pointing to the electrostatic ruthenium(III/II) complex–GOx interaction as one of the most important factors, where the isoelectric point for *A. niger* GOx_{ox} has been reported to be ranged from pH 3.9 to 4.3 [43], and thus GOx_{ox} is negatively charged at pH 7.0. For comparison of the cyclic voltammograms of 0.2 mM $[Ru(NH_3)_5(L^+)]^{3+}$ in 0.1 M phosphate buffer (pH 7.0) in the absence (a) and presence (b) of 0.1 mM GOx_{ox}, all the ruthenium(II) complexes in the presence of GOx_{ox} display the decreases in magnitudes of the peak currents and the same redox potentials as shown in Fig. 4A for $[Ru(NH_3)_5(PymQ^+)]^{3+}$. The decreases in magnitudes of the peak currents suggest that the effective diffusion coefficients of the ruthenium (III/II) complexes may decrease by means of forming adducts with Gox_{ox} as illustrated in Scheme 1. The change in binding constant can be determined according to Eq. (6):

$$E_b{}' - E_f{}' = 0.059\log(K_{Ru(II)}/K_{Ru(III)}) \tag{6}$$

where E_b' and E_f' are the thermodynamic redox potentials for the bound and free complexes, respectively, and $K_{Ru(II)}/K_{Ru(III)}$ is the ratio of binding constants for the $[Ru(NH_3)_5(L^+)]^{3+}$ and $[Ru(NH_3)_5(L^+)]^{4+}$ complexes. For no shift of the redox potential, we calculate $K_{Ru(II)}/K_{Ru(III)}=1$; i.e., the strength for electrostatic binding of $[Ru(NH_3)_5(L_+)]^{3+}$ is the same as that of $[Ru(NH_3)_5(L^+)]^{4+}$ although the charge on both forms is different. The cyclic voltammetric behavior of $[Os(dmbpy)_3]^{2+}$ (dmbpy: 4,4′-dimethyl-2,2′-bipyridine) in the presence of GOx_{ox} was the same as that of $[Ru(NH_3)_5(L^+)]^{3+}$, suggesting that the association of $[Os(dmbpy)_3]^{2+}$ with GOx_{ox} takes place. However, no difference in absorption spectral behavior was observed in the absence and presence of GOx_{ox}. We recently reported that there is only the electrostatic effect between $[Os(dmbpy)_3]^{2+}$ and GOx_{ox} [15]. Fig. 4B shows variation in the intense $d_\pi(Ru^{II}) \rightarrow \pi*(PymQ^+)$ MLCT band of $[Ru(NH_3)_5(PymQ^+)]^{3+}$ with increasing amount of GOx_{ox}. All the MLCT bands of $[Ru(NH_3)_5(L^+)]^{3+}$ exhibit hypochromism and red (bathochromic) shifts (usually attributed to a change of solvent polarity) in the presence of GOx_{ox}, indicating that the pyridinium ions coordinated monodentately to ruthenium may introduce into GOx_{ox} through the active site pocket of ca. 10 Å and act as an antenna that find FAD of the enzyme. It is thus suggested that the importance of the antenna strategy in the electron transfer mediator design; that is, an electron transfer mediator contains a linear wire made of pyridinium ions.

4. Conclusions

In this work, the mediation of dipolar ruthenium(II) ammine complexes containing pyridinium ions $[Ru(NH_3)_5(L^+)]^{3+}$ (L^+: pyridinium ion) in glucose oxidation has been investigated by a voltammetric method. These ruthenium(II) complexes had appropriate redox potentials of 0.10–0.18 V vs. Ag/AgCl that in turn enable the electrode to be polarized at a potential that does not give rise to interfering electrochemical reactions, and high values of k_s, which are the second-order rate constants for electron transfers from GOx_{red} to $[Ru(NH_3)_5(L^+)]^{4+}$, to minimize competition with dissolved dioxygen. The k_s values of $[Ru(NH_3)_5(L^+)]^{3+}$ were greater than those of osmium(II) complexes possessing similar redox potentials which were most commonly used. These facts suggest that the electrostatic ruthenium(III/II) complex–GOx interaction and/or "antenna effect" of pyridinium ions introduced into GOx may contribute to greater k_s values. Consequently, these dipolar ruthenium(II) complexes are concluded to be useful for the electron transfer mediators of amperometric glucose sensors.

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