Electrochemical analysis of the coordination sphere of ruthenium(II) as electron transfer mediator in glucose oxidase catalysis in aqueous solutions

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The redox potentials of the *cis*-$[Ru(LL)_2XY]^{n+}$ complexes (LL = 2,2′-bipyridyl (bpy), 1,10-phenanthroline (phen), and 4,4′-dimethyl-2,2′-bipyridyl (Me_2bpy); X, Y = Cl^-, Br^-, CO_3^{2-}, NO_2^-, SCN^-, N_3^-, H_2O, and DMSO) in aqueous buffer solutions were measured and analyzed in the framework of the Lever theory on the additivity of contributions of ligands (E_L) to the apparent redox potential of the complex ($E^{\circ\prime}$). The complexes manifest the properties of reversible or quasireversible redox systems, whose formal redox potentials lie in the 0.2—0.5 V range. The complexes are efficient electron transfer mediators between the active center of glucose oxidase (GO) from *Aspergillus niger* and an electrode.

Key words: ruthenium complexes, glucose oxidase, electron transfer, cyclic voltammetry, kinetics.

Ruthenium(II) polypyridine complexes[1] are characterized by important properties: a high structural stability and easy reversible electron transfer accompanied by a noticeable change in the color, which is convenient for their use as electron transfer mediators. One of promising directions of the application of the ruthenium(II) polypyridine complexes *cis*-$[Ru(LL)_2XY]^{n+}$ is associated with the development of amperometric glucose biosensors.[2—5] A mediator for glucose amperometric biosensors is selected in such a way that its redox potential lies in the region from 0 to +0.300 V *vs.* SCE. On the one hand, this interval is due to the fact that the electron transfer rate decreases if the potential of the mediator is shifted to the anodic region by less than 0.200 V relatively to the potential of the active center of glucose oxidase, the pair oxidized/reduced flavine adenine dinucleotide ($FAD/FADH_2$), which is accepted to be in neutral solutions[6] −0.221 V SCE. On the other hand, the region of potentials of electrochemically active compounds, which are usually present in analyzed solutions, lies above 0.300 V.

Researchers attempted to find a dependence of the redox potential of the transition metal on its structure, nature, and number of ligands from the middle 1970s. In works,[7,8] the authors proposed the conception of the additivity of contributions of ligands to the redox potential of the whole complex and the formulas for the calculation of the redox potentials $E^{\circ\prime}_{theor}(Ru^{III/II})$ of the ruthenium complexes

$$E^{\circ\prime}_{theor} = 0.97[E_L] + 0.04, \qquad r^2 = 0.99, \qquad (1a)$$

$$[E_L] = 2nE_L(LL) + (6 - 2n)E_L(X),$$

where $E_L(LL)$ are the semiempirical values that characterize the contributions of bidentate ligands, and $E_L(X)$ are the contributions of monodentate ligands to the apparent potential of the complex. Equation (1a) was obtained for solutions of complexes in acetonitrile. The following equation is valid for aqueous solutions:

$$E^{\circ\prime}_{theor} = 1.14[E_L] - 0.35, \qquad r^2 = 0.97, \qquad (1b)$$

where $E^{\circ\prime}_{theor}$ are the redox potentials of the corresponding complexes in neutral aqueous solutions *vs.* saturated calomel electrode (SCE). These correlations describe satisfactorily potentials of a broad series of ruthenium(II/III) complexes in the corresponding solvents and allow the E_L values to be found for many organic and inorganic ligands.

The $[Ru(LL)_3]^{2+}$ complexes (LL = bpy, phen) have previously been used[9—11] to study the intramolecular electron transfer at long distances (for example, in protein molecules). However, too high potentials of these compounds (~1.5 V) *vs.* saturated hydrogen electrode (SHE in acetonitrile) prevent their application as

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mediators in the composition of amperometric biosensors based on FAD-dependent oxidases. According to the Lever conception,[7] the apparent potential ($E^{\circ\prime}_{obs}$) of the $[Ru(bpy)_3]^{2+}$ complex can substantially be decreased by the replacement of 2,2′-bipyridyl by ligands with negative E_L values. These are, *e.g.*, anions of inorganic acids, an NO^+ cation, and some carboxylate anions.

In order to satisfy requirements imposed to biosensors, mediators must be reversible redox pairs, which are insensitive to changes in the pH and ionic strength of the medium and possess high rate constants of electron transfer to minimize the influence of oxygen on the oxidation rate of the reduced form of the enzyme.[12]

In this work, the Lever approach was used, on the one hand, to estimate formal redox potentials and choose necessary complexes and, on the other hand, to predict the composition of the inner coordination sphere of the Ru complexes in aqueous solutions. Based on the calculated redox potentials, we chose and synthesized the following complexes: *cis*-$[Ru(LL)_2X_2]$ (LL = bpy, 1,10-phenanthroline (phen); X = Cl^-, Br^-, SCO_3^{2-}, NO_2^-, NCS^-, N_3^-), *cis*-$[Ru(LL)_2(NO)Cl](BF_4)_2$ (LL = bpy, phen), and *cis*-$[Ru(Me_2bpy)Cl_2]$ (Me_2bpy = 4,4′-dimethyl-2,2′-bipyridyl). These complexes were characterized as mediators for glucose oxidase: their formal redox potentials and the second order rate constants for the oxidation of reduced $FADH_2$ in glucose oxidase in aqueous solutions at 25 °C and pH 7.0 were determined by cyclic voltammetry (CVA).

Experimental

Instruments and materials. The ruthenium(II) complexes were prepared by described procedures: *cis*-$[Ru(LL)_2X_2] \cdot 2H_2O$ (LL = bpy, phen, and Me_2bpy; X = Cl^- and $1/2CO_3^{2-}$), *cis*-$[Ru(bpy)_2X_2]$ (X = Br^-, NO_2^-, NCS^-, N_3^-),[13—15] and *cis*-$[Ru(LL)_2(NO)Cl](BF_4)_2$ (LL = bpy, phen).[16] D-Glucose (ICN) and glucose oxidase with an activity of 270 u mg^{-1} (Sigma) were used. The components of a phosphate buffer (KH_2PO_4 and Na_2HPO_4) with the purity at least analytical grade were used without additional purification. Electrochemical measurements were carried out on an IPC-4 potentiostat-galvanostat equipped with a computer interface (Institute of Physical Chemistry, RAS, Moscow). An electrochemical cell included a pyrographite working electrode, saturated calomel reference electrode, and platinum auxiliary electrode. Before each measurement the working electrode was polished for 10—15 min using diamond (3 μm) and Al_2O_3 (1.0 μm) abrasive pastes.

Methods. Working solutions of the ruthenium(II) complexes with a concentration of 0.4 mmol L^{-1} were prepared by the dissolution of a weighed sample of the complex in the corresponding volume of a 0.01 *M* phosphate buffer (pH 7.0). The initial 1 *M* solution of D-glucose was obtained by the dissolution of a weighed sample of glucose in a phosphate buffer and storing of the resulting solution at ~20 °C for 24 h to achieve the complete mutarotation of D-glucose. A solution of glucose oxidase in distilled water (1.3 mmol L^{-1}) was stored at −20 °C. A solution of the complex was placed into a 8-mL electrochemical cell thermostatted at 25 °C, argon was bubbled through the cell for 30 min, then a solution of the enzyme was added, whose concentration in the cell was 5 mol L^{-1}, and cyclic voltammograms were recorded at the sweep rate from 2 to 100 mV s^{-1} in the 0—1 V potential range. Then the same experiment was conducted after the addition of a fresh solution of D-glucose, whose concentration in the cell was 100 mmol L^{-1}. The data obtained were processed according to the Bourdillon method[17] (see below).

Results and Discussion

Electrochemistry of aqueous solutions of ruthenium(II). The ruthenium(II) complexes *cis*-$[Ru(LL)_2X_2]$ (LL = bpy, 1,10-phen; X = Cl^-, Br^-, NCS^-, SCO_3^{2-}, N_3^-, NO_2^-) or *cis*-$[Ru(LL)_2(NO)Cl]^{2+}$ were studied as possible electron transfer mediators involving GO from *Aspergillus niger*. The Level conception about additive contributions of E_L from different ligands and correlation (1b) were used for the preliminary theoretical estimation of redox potentials of these complexes. This equation was obtained for precisely aqueous solutions of Ru^{II} complexes and takes into account the hydration of the coordination sphere. However, thus calculated E_{theor} potentials differed considerably from E_{obs} measured *vs.* SCE in a 0.01 *M* neutral phosphate buffer at 25 °C. This could be related to a change in the composition of the complex on going from the solid phase to an aqueous buffer solution. To verify this assumption, we analyzed the structure of the complexes dissolved in D_2O using ^1H NMR. It turned out that the $[Ru(LL)_2(NCS)_2]$, $[Ru(LL)_2(CO_3)]$, and $[Ru(LL)_2(DMSO)_2]^{2+}$ compounds in neutral solutions do not change their composition during two weeks. The $[Ru(bpy)_2Cl_2]$ complex undergoes aquation to form mono- and diaqua complexes[18—22] $[Ru(bpy)_2(H_2O)Cl]^+$ and $[Ru(bpy)_2(H_2O)_2]^{2+}$. The pK_a values for *cis*-$[Ru(bpy)_2(H_2O)_2]^{2+}$ are 10.26 [21] or 8.9 [18]; hence, in a neutral aqueous solution the concentration of the diaqua complex much exceeds that of the hydroxoaqua complex. In neutral and alkaline solutions the $[Ru(bpy)_2(NO)Cl]^{2+}$ cation is transformed into $[Ru(bpy)_2(NO_2)Cl]$ followed by aquation and the formation of the $[Ru(bpy)_2(NO_2)(H_2O)]^+$ ion.

Taking into account these changes in the composition of the coordination sphere of the starting complexes, we can see that the Lever conception describes well the experimental values of redox potentials of the complex species in solutions under study. According to Lever′s data, the aqua ligand has virtually no effect on the potential of the complex, $E_L(H_2O) = 0.04$, and chloro- and bromoligands introduce significant negative contributions to the total potential, $E_L(Cl^-) = -0.24$ and $E_L(Br^-) = -0.22$ V. The dependence of the observed redox potentials on the total contributions from the ligands is presented in Fig. 1. The dependence is described with a good accuracy by the equation

$$E_{app} = (0.95 \pm 0.01)\Sigma E_L - (0.25 \pm 0.01), \quad r^2 = 0.98. \qquad (2)$$

However, this equation is poorly fulfilled when the thiocyanate ion is used as ligand and it is inappropriate

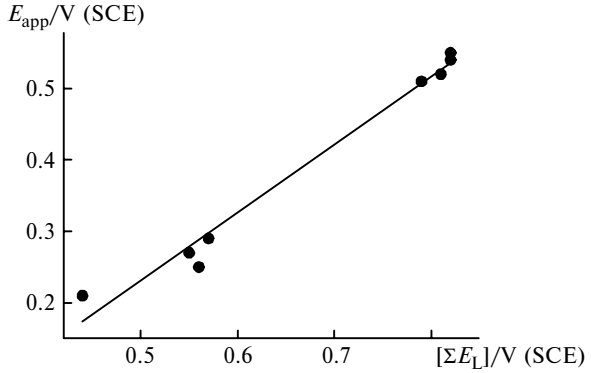

Fig. 1. Plots of the measured redox potentials of the ruthenium complexes *vs.* total contributions from the ligands of the inner coordination sphere.

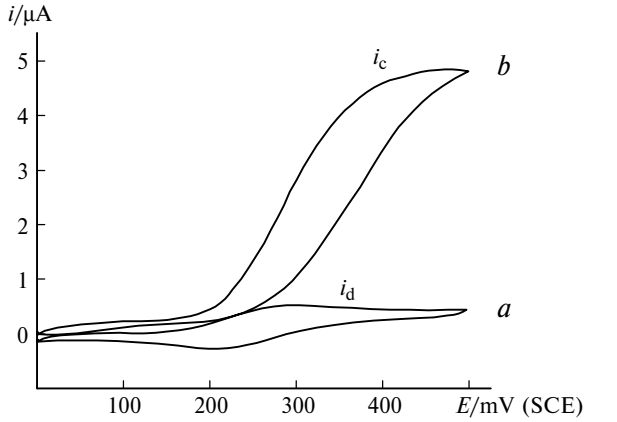

Fig. 2. CVA curves for a $0.4 \cdot 10^{-3}$ *M* solution of $[Ru(Me_2bpy)_2Cl_2]$ in a 0.01 *M* phosphate buffer (pH 7.0 and 25 °C) in the presence of $2.5 \cdot 10^{-6}$ mol L^{-1} GO (*a*), $2.5 \cdot 10^{-6}$ mol L^{-1} GO, and $60 \cdot 10^{-3}$ mol L^{-1} D-glucose (*b*). Sweep rate 10 mV s^{-1}.

for DMSO-containing complexes. Thiocyanate can be linked with the central atom through the nitrogen or sulfur atom, and the DMSO molecule is coordinated, depending on the solvent, also through the sulfur or oxygen atom. Therefore, the E_L parameters for these ligands need, most likely, to be refined. Data on E_L for the carbonate anion are absent from Ref. 6. The E_L value of the carbonate ligand can be calculated from the experimental $E^{\circ\prime}$ values obtained in this work using the equation

$$E_L(CO_3^{2-}) = [\Sigma E_L([Ru(LL)_2CO_3]) - 4E_L(bpy)]/2, \qquad (3)$$

$[Ru(LL)_2CO_3]$ is the inner coordination sphere of the complex

since the inner coordination sphere of the $[Ru(LL)_2(CO_3)]$ complexes does not experience noticeable changes in an aqueous solution and the electrochemical redox process involving these complexes is reversible and not impeded by side reactions.

Thus, the contribution of the carbonate anion *vs.* saturated hydrogen electrode (SHE) is −0.14 V. For comparison, the E_L parameter for the oxalate anion $C_2O_4^{2-}$ is −0.17 V *vs.* SHE.

The $[Ru(bpy)_2(N_3)_2]$ complex is virtually insoluble in a neutral buffer aqueous solution. Therefore, to estimate its redox potential at pH 7.0, its solubility was enhanced by the addition of the nonionogenic surfactant Triton X-100 and acetonitrile. The concentration of the complex in a solution containing from 2 to 10% Triton X-100 was insufficient for the detection of redox peaks by the CVA method. However, this restriction was surmounted in a solution containing 5% acetonitrile instead of Triton X-100. The redox potential of this complex was 0.195 V at a sweep rate of 8 mV s^{-1}, which agrees well with the theoretically calculated value $E^{\circ\prime}_{theor}$ = 0.192 V *vs.* SCE. The complex with azide is an irreversible redox system: the difference of potentials between the anodic and cathodic redox peaks is 110 mV. The cathodic peak is weakly pronounced, and the height of the anodic peak depends on the sweep rate and at 100 mV s^{-1} it shifts to the cathodic region by 0.090 V compared to the potential of the anodic peak at 8 mV s^{-1}.

For the water-soluble complexes, the cyclic voltammograms were obtained at sweep rates from 2 to 100 mV s^{-1}. The cyclic voltammogram of the $[Ru(Me_2bpy)_2Cl_2]$ complex on the pyrographite electrode at pH 7.0 and 25 °C and a sweep rate of 10 mV s^{-1} is presented in Fig. 2, *a*. This CVA curve is characteristic of most studied complexes with the $[Ru(LL)_2XY]$ composition under these conditions. The difference of potentials of the cathodic and anodic peaks of these complexes (ΔE) ranges from 0.065 to 0.090 V, the cathodic and anodic currents are equal, and the position of the half-wave redox potential is independent of the sweep rate. Rigidly speaking, the resolution of the redox peaks of the complexes somewhat exceeds the critical value for the reversible system $\Delta E \approx 2.30RT/nF$ = 0.060 V (where n is the number of electrons, T is the temperature of the system (K), R is the universal gas constant, and F is the Faraday constant), and, in this case, quasi-reversibility is implied. For the $[Ru(bpy)_2(NO_2)(H_2O)]^+$ ion obtained in a solution from the nitroso complex, ΔE = 0.140 V. Due to high redox potentials and pronounced quasireversibility, these complexes cannot be used as electron transfer mediators involving GO.

Electron transfer kinetics. The efficiency of the ruthenium complexes as electron exchange mediators in the "glucose—glucose oxidase (FAD)—electrode" system is characterized by the electron transfer rate. This process can be presented as four reactions[17]

$$\text{FAD} + \text{glucose} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{FAD} \cdot \text{glucose}, \qquad (3a)$$

$$\text{FAD} \cdot \text{glucose} \xrightarrow{k_2} \text{FADH}_2 + \text{glucolactone}, \qquad (3b)$$

$$FADH_2 + 2\ Ru^{3+} \xrightarrow{k_3} FAD + 2\ Ru^{2+}, \quad (3c)$$

$$Ru^{2+} \xrightarrow{-e^-} Ru^{3+}, \quad (3d)$$

where FAD is oxidized flavine adenine dinucleotide, $FADH_2$ is reduced flavine adenine dinucleotide, and FAD · glucose is the "enzyme-substrate" complex.

The rate (V) of the whole process is described by the equation

$$V = D_{Ru^{2+}} \frac{\partial^2[Ru^{2+}]}{\partial x^2} - \frac{2k_3 C_E^{\circ}[Ru^{2+}]}{1 + k_3(k_2^{-1} + (k_{-1} + k_2)k_1k_2[\text{glucose}])[Ru^{2+}]}, \quad (4)$$

where $D_{Ru^{2+}}$ is the diffusion coefficient of the oxidized mediator, $[Ru^{2+}]$ is the equilibrium concentration of the oxidized mediator in a solution (mol L^{-1}), [glucose] is the equilibrium concentration in a solution (mol L^{-1}), C_E° is the total concentration of the active enzyme in a solution (mol L^{-1}), and x is the distance to the electrode (cm).

Stage (3c) is a combination of several processes.[17] However, the Bourdillon method[17] allows the slowest rate constant of this process k_3 to be found from CVA data. This is possible under the condition where, first, the enzyme concentration is much lower than the concentrations of the substrate and co-substrate. Second, the concentration of glucose exceeds much that of the mediator. Then the glucose concentration can be considered unchanged in the whole reaction-diffusion layer and equal to the total concentration, and the enzyme can be considered motionless compared to the mediator because the diffusion coefficient of the latter is much higher than that of the enzyme. According to the proposed conception,[17] the oxidation of $FADH_2$ is a multistage process producing a mediator complex with $FADH_2$ and its incompletely oxidized forms. It is postulated that the rate constants of all stages of decomposition of these complexes are much higher than k_2, which simplifies noticeably the expression for the rate of the whole process (3). The boundary conditions for changing the mediator concentration during the electrochemical process are the following:

at $t = 0$, $x \geq 0$ or $x = \infty$, $t \geq 0$ $\quad [Ru^{3+}] = 0$;

at $x = 0$, $t \geq 0$

$$[Ru^{3+}] = \frac{C^{\circ}_{Ru^{2+}}}{1 + \exp\frac{nF}{RT}(E - E^{\circ})}, \quad (5)$$

where t is the duration of experiment, x is the distance to the electrode (cm), $C^{\circ}_{Ru^{2+}}$ is the total initial concentration of the reduced mediator (mol L^{-1}), E is the current potential (V), and E° is the standard redox potential of the complex (V). The anodic current i_d is proportional to the gradient of the mediator concentration on the electrode surface with the surface area S

$$i_d = -FSD_{Ru^{3+}}\left(\frac{\partial[Ru^{3+}]}{\partial x}\right)_{x=0}, \quad (6)$$

where F is the Faraday constant.

The sweep of electrode potentials in time can be presented as

$$E = E_1 + \upsilon t, \quad (7)$$

where υ is the sweep rate, and E_0 is the initial potential. The current detected during changing the mediator concentration near the electrode surface is described by the equation

$$i = FSC_p^{\circ}\left(\frac{D_{Ru^{3+}}F\upsilon}{RT}\right)^{0.5}\left(\frac{\partial q}{\partial y}\right)_{y=0}, \quad (8)$$

where y is determined by the expression

$$y = x\left(\frac{F\upsilon}{RT}\right)^{0.5}, \quad (9)$$

and q is the normalized mediator concentration equal to the ratio of the equilibrium concentration of the oxidized form of the mediator in a solution to the total concentration of the oxidized and reduced forms of the mediator.

The curves of catalytic anodic currents reach a plateau of the catalytic current i_c. The ratio of this value to the value of the peak of the diffusion anodic current i_d in the CVA curve of the mediator is inversely proportional to the square root of the sweep rate of the potential

$$\frac{i_c}{i_d} = \frac{1}{0.446}\left(\frac{2k_3RT}{F}\right)^{0.5}\sqrt{\frac{C_E^{\circ}}{\upsilon}}. \quad (10)$$

This expression allows the determination of the rate constant k_3 from the tangent slope of the rectilinear region of the plot of i_c/i_d *vs.* $(C_E^{\circ}/\upsilon)^{0.5}$.

The CVA curves for a 0.4 mmol L^{-1} solution of the $[Ru(4,4'\text{-}Me_2\ bpy)_2Cl_2]$ complex in a 0.01 mmol L^{-1} phosphate buffer at pH 7.0 containing 2.5 μmol L^{-1} GO, in the absence and presence of 60 mmol L^{-1} D-glucose, are presented in Fig. 2. The CVA curve does not change in the presence of the enzyme. After the addition of glucose to a mixture of GO and mediator through which argon is bubbled, a catalytic wave is observed, and the peaks disappear completely at sweep rates lower than 20 mV s^{-1}. At rates >100 mV s^{-1} the ratio of the catalytic to diffusion currents tends to unity, and the linear region of the plot is extrapolated to zero in order to obtain the k_3 value (Fig. 3). The amplification factors i_c/i_d are high in the studied series

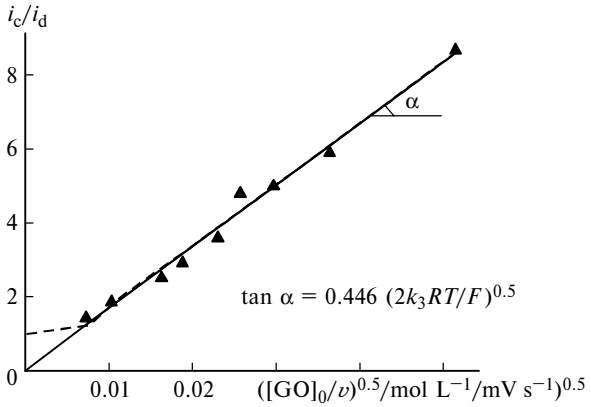

Fig. 3. Plot for the calculation of the rate constant of electron transfer k_3 for the $[Ru(Me_2bpy)_2Cl_2]$ complex in a 0.01 *M* phosphate buffer (pH 7.0 и 25 °C) in the presence of $2.5 \cdot 10^{-6}$ mol L^{-1} GO and $60 \cdot 10^{-3}$ mol L^{-1} D-glucose.

of complexes and reach, in the case of the initial $[Ru(Me_2bpy)_2Cl_2]$ complex, 12—13 at a sweep rate of 2 mV s^{-1}.

The values of the k_3 rate constants determined by formula (6) (Table 1) range from $7.0 \cdot 10^4$ L mol $^{-1}$ s^{-1} for $[Ru(bpy)_2(NO_2)(H_2O)]^+$ to $2.1 \cdot 10^5$ L mol^{-1} s^{-1} for $[Ru(Me_2bpy)_2(H_2O)Cl]^+$. They are somewhat higher than the constants for ferrocenes[6,9,17] widely used in glucose biosensors but lower or at least do not exceed the corresponding values for the osmium complexes[2,3,23] with a high positive charge in the inner coordination sphere: $6.1 \cdot 10^6$ L mol^{-1} s^{-1} for $[Os(Me_2bpy)_2(DA\text{-}bpy)]^{3+}$ (DA-bpy = 4,4′-diamino-2,2′-bipyridyl), $4.0 \cdot 10^5$ L mol^{-1} s^{-1} for $[Os(TEAM\text{-}bpy)_2(DA\text{-}bpy)]^{7+}$ (TEAM-bpy = 4,4′-(*N*,*N*,*N*,-triethylammoniummethyl)-2,2′-bipyridyl). The current theory of electron transfer[23,24] establishes a relationship betwen the rate constant of oxidation of the GO active center and the following factors:

1) emf of the reaction, *i.e.*, the difference between the redox potentials of the mediator and FAD/$FADH_2$ pair;

2) change in the free activation energy, which is determined, in turn, by a change in the reorganization energy, edf of the process, electrostatic interaction of charged particles of reactants, distance between them, and ionic strength of the solution.

A study[17] of the kinetics of glucose oxidation in the presence of glucose oxidase and several ferrocenes indicates the formation of a complex of the mediator with the active center of the enzyme. In several cases, this formation is the limiting stage of electron transfer during the successive oxidation of the $FADH_2$ molecule to FAD. The ruthenium complexes are presumably bound to the His516 and His559 residues localized in the immediate vicinity of the active center of glucose oxidase.[25,26]

Analysis of the data in Table 1 indicates the influence of the structure of the inner coordination sphere on the rate constants of intermolecular electron transfer. However, neither a correlation of the free energies between logk_3 and redox potentials, nor the activating influence of the positive charge of the complex are observed. The highest k_3 value ($2.1 \cdot 10^5$ L mol^{-1} s^{-1}) was found for the $[Ru(Me_2bpy)_2(H_2O)Cl]^+$ complex, which has the lowest redox potential (0.21 V). The main activating factor, most likely, is the electron-donor effect of the methyl substituents in the bipyridyl ligands. By contrast, the $[Ru(bpy)_2(DMSO)_2]^{2+}$, $[Ru(bpy)_2(NO_2)(H_2O)]^+$, and $[Ru(phen)_2(NO_2)(H_2O)]^+$ complexes with the accepting X ligands possess high redox potentials: 0.51, 0.52, and 0.55 V, respectively. However, the k_3 rate constants for these complexes are at most $0.8 \cdot 10^5$ L mol^{-1} s^{-1} and close to those for complexes with lower potentials. Rather high k_3 values are also observed for the $[Ru(bpy)_2(H_2O)Br]^+$ ($1.4 \cdot 10^5$ L mol^{-1} s^{-1}) and $[Ru(bpy)_2(CO_3)]$ ($1.1 \cdot 10^5$ L mol^{-1} s^{-1}) complexes. However, they are much lower for $[Ru(bpy)_2(H_2O)Cl]^+$ and $[Ru(phen)_2(CO_3)]$. Probably, in this case, the influence of the enzyme—mediator complex prevails, and the rate of its formation depends on the exchange rate of the acido ligands in the inner coordination sphere and the size of the mediator molecule.

Note in conclusion that the Lever approach[6] used allowed us to select the ruthenium complexes with a specified potential and, in the case when theoretical and experimental redox potentials do not coincide, to suggest a possible structure of the ruthenium(II) complex under certain conditions. The easy exchange of the acido ligands with solvent molecules or other acido ligands simplifies the synthesis of complexes with the inner coordination sphere with a specified composition and can be used for the modification of the active center of GO by one of the complexes listed, as it has

Table 1. Total contribution of the ligands $[\Sigma E_L]$,[7] calculated and experimental redox potentials in aqueous solutions relatively to SCE, and rate constants k_3 of the ruthenium(II) complexes at pH 7.0 (0.01 *M* phosphate buffer, 25 °C)

Complex	$[\Sigma E_L]$	$E^{\circ\prime}_{theor}$	$E^{\circ\prime}_{obs}$	$k_3 \cdot 10^{-5}$/ L mol^{-1} s^{-1}
		V		
$[Ru(bpy)_2(H_2O)Cl]^+$	0.55	0.28	0.27	(0.71±0.10)
$[Ru(bpy)_2(H_2O)Br]^+$	0.57	0.30	0.29	(1.40±0.10)
$[Ru(bpy)_2(NCS)_2]$	0.67	0.42	0.25	(0.70±0.10)
$[Ru(bpy)_2(N_3)_2]$	*	0.19	0.19	—
$[Ru(bpy)_2CO_3]$	*	*	0.23	(1.10±0.10)
[Ru(4,4′-Me_2bpy)$_2(H_2O)Cl]^+$	0.44	0.15	0.21	(2.10±0.30)
$[Ru(phen)_2(H_2O)Cl]^+$	0.56	0.28	0.25	(0.75±0.10)
$[Ru(phen)_2CO_3]$	*	*	0.24	(0.80±0.10)
$[Ru(bpy)_2(DMSO)_2]^{2+}$	1.73	1.62	0.51	(0.70±0.11)
$[Ru(bpy)_2(NO_2)(H_2O)]^+$	0.81	0.58	0.52	(0.70±0.01)
$[Ru(phen)_2(NO_2)(H_2O)]^+$	0.82	0.58	0.55	(0.80±0.00)

* The contribution of E_L for the carbonate anion was not determined in Ref. 7 because the complex was insoluble.

been done in the case of $[Ru(LL)_2Cl_2]$ (LL = bpy, phen).[25]

Unlike many osmium complexes, ferrocenes, and quinones, the studied ruthenium(II) compounds are well soluble in neutral aqueous media and exist in a solution as positively charged particles. This should accelerate the electron transfer between the active center of GO and the electrode during the enzymatic oxidation of D-glucose. These compounds are quasi-reversible redox systems with potentials satisfying requirements imposed to mediators of glucose oxidase and demonstrate high rate constants of electron transfer in an aqueous solution at pH 7.0 and 25 °C. In addition, the ruthenium compounds are relatively harmless compared to other mediators for glucose oxidase, which is an important point for the creation of glucose biosensors implanted into the human body.

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