Complexes of folic acid, lumiflavin and riboflavin with bis(2,2′-bipyridine)ruthenium(II). Facilitated formation of flavosemiquinone complexes and substantial decrease of pK_a(NH)

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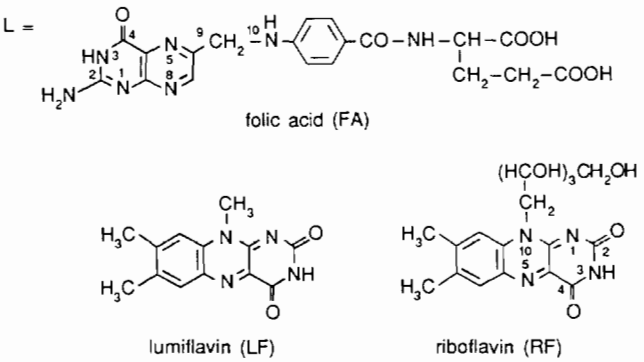
Abstract

Folic acid, lumiflavin and riboflavin bind one $Ru(bpy)_2^{2+}$ complex fragment (bpy = 2,2′-bipyridine). In view of previous results for lumazine and methoxatine complexes, the appearance of characteristically pH-dependent metal-to-ligand charge transfer spectra and a facilitated ligand reduction strongly suggest an α-iminocarbonyl chelate coordination of the metal at positions O(4) and N(5). The pK_a values of the heterocyclic N(3)–H protons are lowered from about 9 to about 5 upon metal coordination.

Introduction

The coordinatively inert [1], yet electronically active ruthenium(II) center in complexes with nitrogen ligands makes metal complex fragments such as $Ru(NH_3)_4^{2+}$ or $Ru(bpy)_2^{2+}$ (bpy = 2,2′-bipyridine) very suitable as probes which bind in a defined 'covalent' manner to biochemically relevant ligands under physiological conditions [1–5]. Previous examples have included tetraammineruthenium complexes of riboflavin, pterins [2] or ascorbate [3] and $Ru(bpy)_2^{2+}$ chelate complexes of cofactor PQQ (methoxatine) [4] and 1,3-dimethyllumazine [5]. In this paper we wish to report the synthesis and characterization of coordination compounds between $Ru(bpy)_2^{2+}$ and two flavins, lumiflavin and riboflavin (vitamin B_2), and the coenzymatic folic acid, a pterin derivative (Scheme 1).

Riboflavin and folic acid are essential cofactors, e.g. for biological electron transfer [6] and for the biosynthesis of neurotransmitters and amino acids [7]; they operate in various metal-containing oxidoreductase enzymes [8, 9] and undergo reversible reduction to semi- and hydroquinonoid forms (flavins [6]) or dihydro and tetrahydro derivatives (folic acid [7]) during their biological function.



Scheme 1.

Experimental

Materials

The starting material, *cis*-dichlorobis(2,2′-bipyridine)ruthenium(II) dihydrate, $Ru(bpy)_2Cl_2 \cdot 2H_2O$, was prepared following literature procedures [10]. Folic acid, lumiflavin and riboflavin were purchased from Fluka and used without further purification. 0.1 N solutions of $HClO_4$ and NaOH for electrochemical and spectroscopic measurements were diluted from 1 M standard solutions (Merck) using bidistilled water.

Synthesis

$Ru(bpy)_2(FA)(PF_6)_2$

96.9 mg $Ru(bpy)_2Cl_2 \cdot 2H_2O$ (0.18 mmol) and 88.3 mg folic acid (0.20 mmol) were dissolved in 50 ml ethanol/water (1:5) and refluxed for 4 h. No unreacted

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$Ru(bpy)_2Cl_2$ was detected in the solution by UV–Vis spectroscopy. The mixture was cooled to room temperature and the product precipitated by addition of 65 mg (0.39 mmol) solid NH_4PF_6. After refrigerating the sample overnight the precipitate was filtered off, washed with ethanol/water and dried at 80 °C. Yield 61.4 mg (30%). *Anal.* Calc. for $C_{39}H_{35}F_{12}N_{11}O_6P_2Ru$: C, 40.94; H, 2.97; N, 13.47. Found: C, 41.86; H, 3.08; N, 13.79%.

$Ru(bpy)_2(LF)(PF_6)_2 \cdot CH_3CN$

52.0 mg $Ru(bpy)_2Cl_2 \cdot 2H_2O$ (0.10 mmol) and 26.1 mg lumiflavin (0.10 mmol) were suspended in 30 ml ethanol/water (1:5) and refluxed for 5 h. After cooling to room temperature no undissolved material remained and UV–Vis spectroscopy indicated no unreacted $Ru(bpy)_2Cl_2$ in the solution. 32.8 mg (0.2 mmol) solid NH_4PF_6 were added to the mixture which was then evaporated to dryness. The solid residue was dissolved in CH_3CN and precipitated with diethyl ether. After refrigerating the mixture overnight the precipitate was filtered off, washed with ether and dried at 60 °C. Yield 70 mg (70%). *Anal.* Calc. for $C_{35}H_{31}F_{12}N_9O_2P_2Ru$: C, 42.00; H, 3.10; N, 12.60. Found: C, 42.17; H, 3.04; N, 12.38%.

$Ru(bpy)_2(RF)(PF_6)_2 \cdot 1/2KPF_6$

50.0 mg $Ru(bpy)_2Cl_2 \cdot 2H_2O$ (0.09 mmol) and 38.8 mg riboflavin (0.10 mmol) were dissolved in 30 ml ethanol/water (1:5) and refluxed for 2 h. After that time there was no unreacted $Ru(bpy)_2Cl_2$ left in the solution as indicated by UV–Vis spectroscopy. The solution was cooled to room temperature and a small excess of solid KPF_6 was added. The volume was reduced by about 1/2, the resulting precipitate was filtered off, washed with small quantities of ethanol/water and dried at 80 °C. Yield 30 mg (28%). *Anal.* Calc. for $C_{37}H_{36}F_{15}K_{0.5}N_8O_6P_{2.5}Ru$: C, 37.92; H, 3.07; N, 9.56. Found: C, 37.83; H, 2.96; N, 9.32%.

Methods and instrumentation

The p*K* values were determined spectroscopically. Equally concentrated ($\approx 10^{-4}$ M) solutions of the complexes were prepared in 0.1 N $HClO_4$ and 0.1 N NaOH. The basic solution was then titrated with the acidic one, measuring pH values and UV–Vis spectra after each addition. pH values were measured with a Knick MultiCalimatic pH meter model 764 equipped with a Schott combination electrode. UV–Vis spectra were recorded with a Shimadzu UV-160 spectrophotometer. The change of absorbance at three different wavelengths was used to calculate the p*K* data using a simplex curve fitting program.

Electrochemical data were measured with a EG&G Princeton Applied Research model 363 potentiostat/galvanostat controlled by a EG&G PAR model 175 universal programmer, using a glassy carbon working electrode, a platinum auxiliary electrode and Ag/AgCl as reference. ESR spectra of electrogenerated species were recorded on a Bruker ESP300 system.

Results and discussion

Complexes $(L)Ru(bpy)_2^{2+}$ could be prepared as bis(hexafluorophosphate) salts from *cis*-$Ru(bpy)_2Cl_2$ in refluxing ethanol/water mixtures. None of the synthetic difficulties reported recently [5b] were encountered under these conditions. Both the pterin moiety in folic acid and the flavin nucleus in lumiflavin and riboflavin have in common

(i) a reducible π system [6, 7] which contains

(ii) a particularly π electron deficient α-iminocarbonyl chelation site O(4)=C–C=N(5) [3, 5, 11] and

(iii) a relatively acidic ($pK_a \approx 9$) NH proton in the pyrimidine ring [2, 6, 7, 12].

The π-accepting α-iminocarbonyl site in flavins, pterins or lumazines is very suitable in binding π electron rich metal centers such as Ru(II), Ag(I), Cu(I) or Re(I) [2, 3, 5, 10, 13]. It is thus not unreasonable to assume that the complexes of $Ru(bpy)_2^{2+}$ with the ligands in Scheme 1 can also have the divalent metal coordinated in a five-membered chelate ring to O(4) and N(5). The $-CH_2-NH-$ part of the side chain of folic acid is sufficiently flexible to allow unhindered coordination at O(4) and N(5).

Cyclic voltammetry in 0.1 M perchloric acid or 0.1 M NaOH solution shows reversible flavin-based reductions $(L)Ru(bpy)_2^{2+} \rightarrow (L^{-\cdot})Ru(bpy)_2^{+\cdot}$ at very similar potentials for the two flavin complexes (Fig. 1, Table 1). The relatively large differences between the

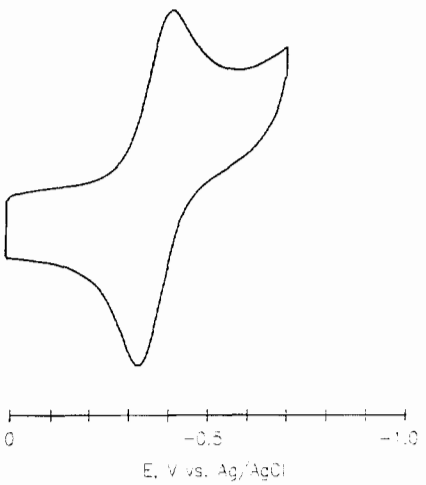

Fig. 1. Cyclic voltammogram of $Ru(bpy)_2(LF)(PF_6)_2$ in 0.1 N $HClO_4$. Reference electrode Ag/AgCl, scan rate 100 mV/s.

TABLE 1. Summary of spectroscopic and electrochemical data

	$Ru(bpy)_2(FA)(PF_6)_2$	$Ru(bpy)_2(LF)(PF_6)_2$	$Ru(bpy)_2(RF)(PF_6)_2$
UV–Vis (λ_{max} (nm))			
0.1 N $HClO_4$			
MLCT(bpy)	353sh	385sh	385sh
	438	411	407
		485	470sh
		516sh	512sh
MLCT(L)	513	644	642
0.1 N NaOH			
MLCT(bpy)	385	399	356
	450sh		395sh
		450	447
		475sh	475sh
		515sh	510sh
MLCT(L)	471	621	614
pK_a (N(3)–H)			
	4.3 ± 0.1	5.2 ± 0.1	5.4 ± 0.1
	(FA: 8.38 [11])	(LF: 10 [2])	(RF: 10.0 [2])
CV (E, V vs. Ag/AgCl)	E_{pa}(ox)	$E_{1/2}$(red)	$E_{1/2}$(red)
0.1 N $HClO_4$	+0.73 (irr.)	−0.29	−0.37
	+0.95 (irr.)		
0.1 N NaOH		−0.81	−0.85

$E_{1/2}$ values in acid and base already suggest a coupling between an acidic site and the electro-/chromophore. A Ru(II/III) couple could not be observed for the flavin complexes below +1.0 V; the tetraammineruthenium complex of riboflavin showed such a wave at +0.89 V [2c] and the number for the $(bpy)_2Ru^{2+}$ analogue should be substantially higher (cf. below). No reduction was observed for the complex of folic acid because the pterin ring is significantly more electron rich than the flavin or lumazine π system [5, 7]. On the other hand, two oxidation waves appear below +1 V which are attributed to irreversible oxidation of the aromatic amine part of folic acid (N(10)) and to the Ru(II/III) transition. A difference of about 0.3 V has been reported recently for the Ru(II/III) couple between pterin and flavin coordinated $Ru(NH_3)_4^{2+}$ complexes [2c]; in contrast to the flavins [2b, 6, 11], the pterins are quite basic ligands even in the oxidized state.

Flavosemiquinones [6] and their complexes [10] often exhibit characteristic ESR spectra; in analogy to previously [14a, b] reported Ru(II) anion radical complexes the lumiflavinsemiquinone complex of $[Ru(bpy)_2]^{2+}$ exhibits a poorly resolved spectrum at $g = 2.0016$.

In the oxidized form, the highly coloured complexes exhibit absorption spectra (Fig. 2, Table 1) with long-wavelength bands which can be attributed to metal-to-ligand charge transfer (MLCT) transitions, i.e. transitions from filled d orbitals of the relatively low-valent metal to unoccupied π^* MOs of the unsaturated ligands bpy [4, 14c] and L [2a, b].

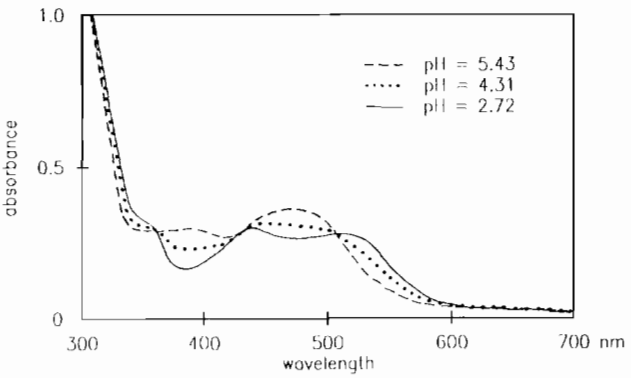

Fig. 2. UV–Vis spectra of $Ru(bpy)_2(FA)(PF_6)_2$ as a function of pH (6×10^{-5} M solution in H_2O).

Transitions $d(Ru) \rightarrow \pi^*(L)$ are found at lower energies than the $d(Ru) \rightarrow \pi^*(bpy)$ transition with its typical maximum around 23 000 cm^{-1} [4, 5, 12c]; both pterins and flavins are better π acceptors than 2,2′-bipyridine ($E_{red} = -2.1$ V). The values for $[Ru(bpy)_2(FA)]^{2+}$ are virtually identical to those of the $Ru(bpy)_2$ complex with 1,3-dimethyllumazine (511 and 432 nm [5a]), indicating an identical chromophore (coordination at O(4) and N(5)). The visible absorption spectra of the flavin complexes are distinguished by additional bands due to intraligand transitions of the tricyclic isoalloxazine ring of flavins [2a, b, 6]. The MLCT bands were found to be pH dependent, shifting to higher energies in neutral and basic solutions; very basic conditions lead to the decomposition of the compounds. While the free

ligands L have pK_a values around 9 for the NH proton in the pyrimidine ring, the acidity is considerably higher for the complexes by about four pK units (Table 1). Although the ionization of the remote, non-conjugated carboxylic groups in folic acid does not affect the MLCT chromophore, both the hypsochromic shift and the substantially lowered pK_a suggest considerable electronic coupling between the metal chelate ring and the NH acidic site. This comparatively [4] strong response of the pK_a(NH) on coordination of a dipositively charged metal complex fragment is mainly due to the vicinity of the two sites, the acidic NH group being α to the metal-coordinating carbonyl group (secondary amide function, 1); coupling via the extended π system of the ligand may contribute also. Remarkably, the effect of $Ru(bpy)_2^{2+}$ is stronger than that of $Ru(NH_3)_4^{2+}$ ($pK_a = 7.4$ for the riboflavin complex in water [2c]).

(1)

The hypsochromic shift of the long-wavelength MLCT band upon ligand deprotonation is a result of the strong destabilization of π^*(L); the π^*(bpy) orbitals are not affected so that a slight destabilization of the ruthenium d orbitals through the ligand field effect leads to a small bathochromic shift for the second MLCT transition $d(Ru) \rightarrow \pi^*(bpy)$.

The redox potentials $E_{1/2(ox)}$ or $E_{1/2(red)}$ which are not observable in aqueous solution can be estimated via formula (2) using the experimental MLCT absorption energies $E_{(op)max}$ (in eV) and the established number of $\chi \approx 0.2$ (e)V for the Franck–Condon contributions from intra- and intermolecular reorganization [14c, 15].

$$E_{(op)max} = \chi + [E_{1/2(ox)} - E_{1/2(red)}] \quad (2)$$

The ruthenium-based oxidations of the flavin complexes are thus calculated to occur around +1.4 V in acidic and +1.0 V in basic solution; the reduction potential of the complex with folic acid is estimated at −1.2 V versus Ag/AgCl in 0.1 M $HClO_4$.

Finally, the one-electron reduced forms of the flavin complexes do not exhibit a long-wavelength MLCT band, instead, the typical absorption features of anionic flavosemiquinones [16] overlap with $d(Ru) \rightarrow \pi^*(bpy)$ bands in the region between 400 and 500 nm.

Summarizing, we have shown that O(4), N(5)-coordinated flavin complexes of $[Ru(bpy)_2]^{2+}$ can be obtained without difficulty as reducible and acidic species. The very similar behaviour of the corresponding folic acid complex in comparison to flavin and lumazine analogues also suggests O(4), N(5) coordination to form a five-membered chelate ring between an electron-rich metal and an unsaturated π-acceptor site. In contrast, the alternative five-membered ring chelation at N(5) and N(10) which is relevant to biological C_1 chemistry [7] requires a special conformation of the side chain, a reduced state of the pterin and an electron-poor metal center.

Acknowledgements

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