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The Design of Metal Chelates with a Biologically Related Redox-Active Part: Conjugation of Riboflavin to Bis(2-pyridylmethyl)amine Ligand and Preparation of a Ferric Complex

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Reaction of a twofold molar excess of bis(2-pyridylmethyl)-amine DPA with 8α-bromoAc4riboflavin in dry DMF over 24 h affords the 8α-[bis(2-pyridylmethyl)amine]-*N*-Ac_4riboflavin ligand. This compound can be described as a tweezer in which the flavin moiety acts as a potential electron mediator. The trichloroferric complex has been prepared, and together with the low potential reduction wave of the flavin moiety, the presence of a reversible Fe^{III}/Fe^{II} couple at a higher potential is observed. The deacetylated ligand 8α-[bis(2-pyridylmethyl)amine]-*N*-riboflavin can easily be obtained.

Introduction

One of the most fascinating aspects of chemical sciences is the way chemists can affect Nature's legacy by modification of biological material, especially when going as far as to design artificial proteins or enzymes that lead to new and sometimes unexpected reactivities.[1–4]

Flavin analogues are well-known as versatile catalysts and are involved in various reactions such as oxidation of tertiary amines, sulfides and ketones, generally with the use of H_2O_2 as the oxidant;[5–7] some cases of oxidation by molecular oxygen have also reported.[8] The idea of linking flavin derivatives to biological compounds in order to access artificial proteins is not new: early examples in which flavins were covalently linked to small proteins like papain for instance encountered some success in the oxidation of dihydronicotinamides.[4] In that case, the flavin fragment acted as the site where the reaction took place. Flavins and derivatives, however, are also known as major electron mediators in biological oxidation processes. It is therefore tempting to associate these derivatives with systems that require electron delivery. First attempts to link flavin derivatives with hemes were reported a long time ago, with an example of a semisynthetic hydroxylase that displayed P-450 activity without a reductase.[9] More recently, covalent binding between a riboflavin and protoporphyrin IX allowed reductive activation of dioxygen by an artificial myoglobin.[10] In these examples, the porphyrin/flavin binds the protein through the porphyrin side. Flavin derivatives, however, can bind proteins by themselves. The ability of riboflavin to bind low potential electron carriers, such as flavoproteins for instance, to produce stable complexes is now well-documented.[11–13] The fact that the flavin/flavodoxin complex can function as a one-electron carrier at a potential close to that of the hydrogen electrode[13] makes the design of flavin appended ligands that can accommodate a redox-active metal highly desirable in order to carry out further redox-assisted chemistry.

For several years now, we have been studying dichloroferrous complexes of pyridylmethyl-amine containing ligands, and we have reported cases of fast biomimetic reactions of dioxygen with the metal centre.[14–16] The end product recovered after oxygenation of the ferrous complex can sometimes be reduced back to ferrous, which prefigures the metal centre for further redox chemistry. It was thus tempting to prepare a system in which a pyridylmethylamine-containing ligand would be covalently attached to a riboflavin derivative, the latter acting here as a redox mediator. Examples of substituted DPA ligands with electron mediators can be found in the literature.[17,18] However, those mediators are synthetic compounds and, to the best of our knowledge, there is currently no example of conjugation with a universal and biologically relevant mediator such as riboflavin. We thus report in this communication the first example of synthesis and characterization of such a ligand, 8α-[bis(2-pyridylmethyl)amine]-*N*-riboflavin, as well as the preparation of an air-stable trichloroferric complex of the acetylated precursor. In addition to standard analytical methods used in synthesis, both acetylated precursor and complex have been characterized by cyclic voltammetry. As a result,

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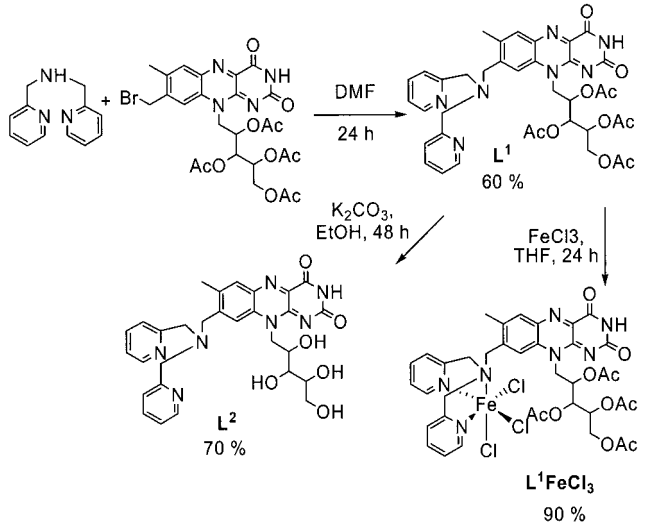
riboflavin can indeed be considered as a redox active mediator in future artificial constructions built around such compounds.

Results and Discussion

As depicted in Scheme 1, ligand L^1 8α-[bis(2-pyridylmethyl)amine]-*N*-Ac_4riboflavin was prepared by reaction of a twofold molar excess of bis(2-pyridylmethyl)amine DPA[19] with 8α-BromoAc_4riboflavin[20] in dry DMF over 24 h, by adaptation of the procedure published for the preparation of 8α-*N*-imidazolylriboflavin.[21] The brownish–orange solid obtained upon crystallization from a CH_2Cl_2/Et_2O solution is a stable compound and was obtained in a 60% yield. Deprotection of the acetyl groups was performed by conventional methods with K_2CO_3 in dry EtOH at room temperature within 48 h. The solution was then neutralized with Amberlite IR–120 exchange resin and afforded, upon concentration, L^2 as a brownish solid moderately soluble in most organic solvents.



Scheme 1. Access to the DPA-riboflavin derivatives L^1, L^2 and ferric complex L^1FeCl_3.

L^1 and L^2 were characterized by mass spectroscopy, elemental analysis and ^{1}H- and ^{13}C NMR spectroscopy. Complex L^1FeCl_3 was prepared by stirring ligand L^1 with an equimolar amount of anhydrous $FeCl_3$ under an inert atmosphere in dry THF (although L^1FeCl_3 is air stable). Following a standard workup,[22] a brownish–yellow solid was obtained in 90% yield. In the ^{1}H NMR spectrum, L^1FeCl_3 displayed extremely broad resonances at δ = 108 and 93 ppm (mid-intensity peak width, 1800 and 1500 Hz, respectively – signals accounting for the DPA moiety[22]), which is in line with a high-spin state for the metal. The flavin part lies further away from the paramagnetic metal than the DPA moiety and related signals appear as broadened resonances between 12 and 3 ppm. The complex was also characterized by mass spectroscopy, elemental analysis, and UV/Vis spectroscopy. The second absorption at λ = 327 nm exhibited a molecular extinction coefficient (ε = $11.31 \cdot 10^3$ $mmol^{-1} \cdot cm^2$) significantly higher than that of the free organic compounds (ε = 7.72 and $5.83 \cdot 10^3$ $mmol^{-1} \cdot cm^2$ for L^1 and L^2, respectively), which indicates the presence of an extra LMCT component due to coordination of the ferric ion.[22]

The cyclic voltammogram (CH_3CN, $TBAPF_6$ 0.1 M, SCE as reference electrode, 100 mV/s – all traces displayed in Figure 1) of L^1 exhibited one reversible reduction wave at $E_{1/2}$ = –0.726 V (Δ_E = 130 mV) and is assigned to the reduction of the acetylated riboflavin moiety [the Ac_4riboflavin recorded as a blank underwent reversible reduction at $E_{1/2}$ = –0.770 V (Δ_E = 50 mV) under the same conditions]. In oxidation, an irreversible anodic signal was found at 1.183 V, which corresponds to the oxidation of the aliphatic amine of the DPA moiety. Upon metallation, this latter signal completely disappeared and a new reversible wave, which accounts for the L^1Fe^{III}/L^1Fe^{II} couple, was observed at $E_{1/2}$ = 0.002 V (Δ_E = 85 mV). For comparison, a reversible couple has already been reported for $DPAFeCl_3$ at $E_{1/2}$ = –0.019 V.[23]

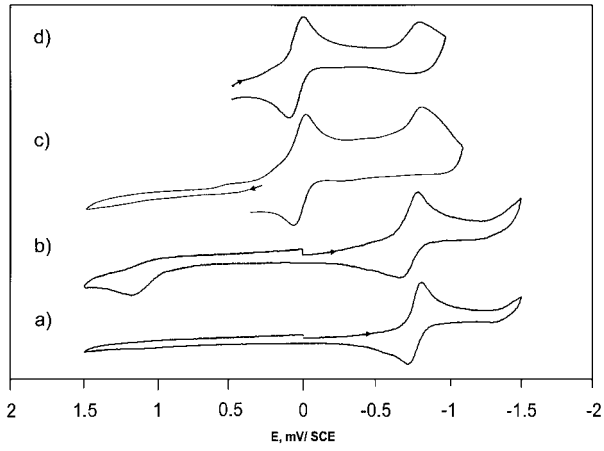


Figure 1. Cyclic voltammograms (CH_3CN, $TBAPF_6$ 0.1 M, SCE as reference electrode, 100 mV/s) of: a) tetraacetyl riboflavin; b) ligand L^1; c) complex L^1FeCl_3; d) detail of L^1FeCl_3 at 200 mV/s.

The reduction of the acetylated riboflavin fragment was found at E_c = –0.838 V, with a signal pattern strongly suggestive of aggregation of the reduced compound at the electrode. Scanning up to 1000 mV/s allowed improvement of the reversibility pattern; however, it remained slightly different from what was expected for an ideally reversible couple. From Figure 1d, the potential is estimated to $E_{1/2}$ = –0.804 V (Δ_E = 68 mV), that is cathodically shifted by 78 mV with respect to L^1. This difference may reflect the presence of a ferrous centre close to the flavin side.

To summarize, a ligand system in which a ubiquitous redox mediator – riboflavin, that is vitamin B2 – is covalently linked to a tridentate nitrogen chelate is now available. A trichloroferric complex with a reversible Fe^{III}/Fe^{II} redox couple can easily be obtained. All compounds reported are stable and easy to handle. We thus believe that the syntheses reported in this communication represent a significant step towards the preparation of functional artificial systems with

DPA-based complexes in which the active metal-containing site is linked through the flavin part to proteins. This approach is currently under investigation in our laboratory.

Experimental Section

^{1}H- and ^{13}C NMR spectroscopic data were recorded with a Bruker AC 300 spectrometer and chemical shifts are standardized to the residual signal of the solvent as a reference. UV/Vis spectra were recorded with a Varian Cary 05 E UV/Vis NIR spectrophotometer. Cyclic voltammetry measurements were obtained with a EG&G PAR 173A potentiostat in a 0.1 M acetonitrile solution of $TBAPF_6$ (supporting electrolyte), with platinum electrodes and saturated calomel electrode as reference. Traces are given in the text. Elemental analyses were carried out by the Service Central d'Analyses du CNRS in Vernaison, France. Mass spectroscopy was carried out with a MicroTOF instrument in the positive mode.

Preparation and Characterization of 8α-[Bis(2-pyridylmethyl)amine]-*N*-Ac$_4$riboflavin (L^1): Bis(2-pyridylmethyl)amine (5 g, 25.2 mmol) in a solution of DMF (10 mL) was added to a solution of 8α-BromoAc$_4$riboflavin (8 g, 12.8 mmol) in DMF (100 mL). The mixture was stirred for 24 h at ambient temperature. DMF was then removed in vacuo, and the resulting oil was dissolved in CH_2Cl_2 (50 mL). This phase was washed twice with distilled water. The organic phase was dried with magnesium sulfate, reduced to 15 mL and a brownish–yellow compound was obtained as a solid by the addition of cold diethyl ether (100 mL). Upon drying, compound L^1 was obtained (5.75 g, 60%). Further recrystallization yielded a compound for which the elemental analysis was satisfactory. L^1·2H$_2$O: $C_{37}H_{43}N_7O_{12}$ (777.30): calcd. C 57.14, H 5.53, N 12.61; found C 56.83, H 5.11, N 12.57. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.69 (d, J = 4.0 Hz, 2 H, DPA α), 8.53 (s, 1 H, NH), 7.92 (s, 1H Ar), 7.71 (t, J = 7.6 Hz, 2 H, DPA γ), 7.56 (s, 1 H, Ar), 7.46 (d, J = 7.6 Hz, 2 H, DPA β′), 7.23–7.19 (m, 2 H, DPA β), 5.59 (d, 1 H, ribityl CH), 5.46 (m, 2 H, ribityl CH_2), 5.3–5.1 (m, 2 H, N-CH_2), 4.44–4.39 (dd, J = 2.2–1.8 Hz, 1 H, aliphatic), 4.31–4.23 (m, 1 H, aliphatic), 4.11–3.98 (dd, J = 14.0–13.9 Hz, 4 H, CH_2-DPA), 3.82 (s, 2 H, CH_2-ribofl.), 2.55 (s, 3 H, methyl), 2.42 (s, 3 H, acetyl), 2.32 (s, 3 H, acetyl), 2.26 (s, 3 H, acetyl), 2.19 (s, 3 H, acetyl) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.6–169.4 (4 acetyl C=O), 159.4 (flavin C=O), 157.8 (2 C_{quat}), 154.7 (flavin C=O), 150.6 (C_{quat}), 149.4 (2 $CH_{pyridyl}$), 148.0 (C_{quat}), 136.5 (2$CH_{pyridyl}$), 134.5 (2 C_{quat}), 132.9 (2$CH_{pyridyl}$), 123.5 ($CH_{pyridyl}$), 122.6 ($CH_{pyridyl}$), 115.5 (2 CH_{Ar}), 70.4, 69.4, 69.1 (3 CH), 61.9 (CH_2 ribofl.), 59.7 (2 $CH_{2pyridyl}$), 55.1 (CH_2), 45.0 (CH_2), 21.4, 21.1, 21.0, 20.8, 20.7 (CH_3) ppm. UV/Vis: λ (ε, 10^3 mmol·cm^2) = 443.6 (9.47), 342.0 (7.72), 268.8 (24.94) nm. MS (ES$^+$, CH_3CN + 0.1% formic acid): m/z = 742.32 [M + H]$^+$.

Preparation and Characterization of Complex L^{1}FeCl$_3$: The procedure outlined in ref.[22] was followed. To a solution of 8α-[bis(2-pyridylmethyl)amine]-*N*-Ac$_4$riboflavin (L^1; 100 mg, 0.135 mmol) in dry THF (30 mL), was added a solution of anhydrous $FeCl_3$ (22 mg, 0.135 mmol) in dry THF (20 mL). Although the compounds are air stable, the reaction was carried out by using Schlenk tubes and cannula. The mixture was stirred for 24 h, and the solvent was then evaporated. The brownish solid was dissolved in CH_3CN, filtered and the solution was concentrated (rotary evaporator). Addition of dry diethyl ether allowed precipitation of L^{1}FeCl$_3$, as a brown, air-stable solid. After washing with diethyl ether and drying, the product was obtained (110 mg, 90%). The compound was recrystallized from acetonitrile/diethyl ether several times. L^{1}FeCl$_3$·4H$_2$O: $C_{37}H_{47}N_7O_{14}FeCl_3$ (974.16): calcd. C 45.51, H 4.81, N 10.04; found C 45.17, H 4.47, N 10.12. ^{1}H NMR (300 MHz, CD_3CN): δ = presence of two very broad signals at 108 and 93 ppm (mid-intensity peak width 1800 and 1500 Hz, respectively). Weak signal at δ = 52 ppm with mid-intensity peak width of 600 Hz. Presence of moderately broadened signals between 3 and 12 ppm. UV/Vis: λ (ε, 10^3 mmol·cm^2) = 445.3 (8.57); 326.9 (11.31); 267.0 (26.78) nm. MS (ES$^+$, CH_3CN = 0.1% formic acid) m/z = 927.07 [M + Na]$^+$, 890.13 [M + Na – Cl]$^+$.

Preparation and Characterization of 8α-[Bis(2-pyridylmethyl)amine]-*N*-riboflavin (L^2): To a solution of potassium carbonate (3 g, 21.3 mmol) in absolute ethanol (150 mL), 8α-[bis(2-pyridylmethyl) amine]-*N*-Ac$_4$riboflavin (L^1; 3 g, 4.3 mmol) was added. The mixture was stirred for 48 h at ambient temperature. After neutralization with AMBERLITE IR-20 resin, the solution was filtered and concentrated in vacuo until a brownish solid precipitated. The solution was washed several times with ether and dried in vacuo. The compound was dissolved in DMF and precipitated by the addition of diethyl ether. The solid was dried in vacuo to afford the product (1.75 g, 70%). Microcrystals of this compound could be obtained from an ethanolic solution by slow evaporation of the solvent. These were kept for elemental analysis and NMR spectroscopy. The ^{1}H NMR spectrum is poorly defined, especially in the aliphatic area. This might be due to agitation of the ribityl moiety and interaction with residual water of the DMSO. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.6–11.3 (4 H, OH), 10.32 [OH (EtOH)], 8.73 (d, J = 4.4 Hz, 2 H, DPA α), 8.31 (s, 1 H, NH), 8.04 (m, 2 H, DPA β), 7.91 (s, 1 H, Ar), 7.85 (s, 1 H, Ar), 7.72 (d, J = 6.2 Hz, 2 H, DPA β′), 7.56 (m, 2 H, DPA γ), 5.20–4.10 (broad signal, ribityl protons and residual water of the DMSO), 4.19 (broad, 2 H, CH_2), 3.65 (broad, 4 H, DPA CH_2), 3.44 [q, J = 7.1 Hz, CH_2 (EtOH)], 2.47 (s, 3 H, CH_3), 1.05 [t, J = 7.1 Hz, CH_3 (EtOH)] ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO) δ = 159.4 (flavin C=O), 157.8 (2 C_{quat}), 154.7 (flavin C=O), 150.6 (C_{quat}), 149.4 (2 $CH_{pyridyl}$), 148.0 (C_{quat}), 136.5 (2 $CH_{pyridyl}$), 134.5 (2 C_{quat}), 132.9 (2 $CH_{pyridyl}$), 123.5 ($CH_{pyridyl}$), 122.6 ($CH_{pyridyl}$), 115.5 (2 CH_{Ar}), 74.0, 73.2, 69.1 (3 CH), 63.8 (CH_2), 63.7 (2 $CH_{2pyridine}$), 58.2 (4 CH_{2EtOH}), 56.4 (CH_2), 50.1 (CH_2), 21.2 (CH_3), 19.2 (4 CH_{3EtOH}). UV/Vis: λ (ε, 10^3 mmol·cm^2) = 447.0 (6.85), 344.7 (5.83), 269.5 (19.03). MS (ES$^+$, CH_3CN/H_2O + 0.1% formic acid): m/z = 574.24 [M + H]$^+$. L^2·4EtOH: $C_{37}H_{55}N_7O_{10}$ (757.40): calcd. C 58.65, H 7.26, N 12.94; found C 58.37, H 7.60, N 12.30.

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