MAGNETIC RESONANCE IN CHEMISTRY
Magn. Reson. Chem. 2001; **39**: 615–620

High sensitivity of the fluorine NMR signals of difluorovinyl analogs of natural hemin: reconstituted heme proteins and self-exchange electron transfer in model compounds

Christine Poliart,[1] Jean-Francois Briand,[1] Frédérique Tortevoie,[1] Jacques Leroy,[2] Gérard Simonneaux[1] and Arnaud Bondon[1]*

[1]Laboratoire de Chimie Organométallique et Biologique, UMR CNRS 6509, Université de Rennes 1, Campus de Beaulieu, 35042 Rennes Cédex, France
[2]Département de Chimie, UMR CNRS 8640, Ecole Normale Supérieure, 75231 Paris Cédex 05, France

Received 1 December 2000; Revised 14 April 2001; Accepted 23 May 2001

The chemical shifts of fluorine in difluorovinyl deuteroporphyrin iron complexes were shown to be very sensitive to the spin state of the metal and the nature of the ligand(s). Reconstituted myoglobin was used as a model heme protein with an exchangeable heme. Large variations in the fluorine chemical shifts in both the ferric and ferrous states were observed. This strong sensitivity to the nature of the metal ligand and the structural resemblance to natural hemin make this fluorinated porphyrin a good probe for the study of heme proteins. The large variations of chemical shifts depending on the oxidation state also permitted the measurement of the electron self-exchange rate constants of bis(1-methylimidazole)iron complexes in various solvents by analysis of line broadening of the ^{19}F NMR signals. The experimental rate constants were strongly affected by the nature of the solvent, varying from 3.9×10^7 to 24.1×10^8 mol l^{-1} s^{-1} for DMSO-d_6 and acetone-d_6, respectively. The solvent parameters were used to estimate the outer-sphere reorganization energies. The experimental rate constants in chloroform and in DMSO are in good agreement with these calculated outer-sphere reorganization energies. Copyright © 2001 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{19}F NMR; fluorinated porphyrins; heme; paramagnetic; heme proteins; electron transfer

INTRODUCTION

Incorporation of fluorine atoms in biological molecules permits a dramatic simplification of the spectroscopic data thanks to ^{19}F NMR. This advantage has been explored with different systems such as biochemical tracers, mechanistic probes and markers of structural or dynamic changes.[1–3] Several strategies can be used for the preparation of fluorine-containing peptides or proteins.[3] One attractive way, for proteins bearing prosthetic groups, is to reconstitute the apoprotein with a fluorinated prosthetic moiety. Many heme proteins contain a removable heme which can be easily replaced by such a fragment.

Extensive studies have been devoted to the synthesis of β-fluorinated pyrroles: 3-fluoropyrrole and 3,4-difluoropyrrole, 3-trifluoromethylpyrrole and 3,4-bis(trifluoromethyl)pyrrole.[4–11] β-Octafluoro(*meso*-tetraaryl)porphyrins, recently prepared from 3,4-difluoropyrrole and benzaldehydes,[12,13] have been used as biomimetic catalysts.[14,15] In addition, several other synthetic porphyrins have also been reported.[7,16–18] As far as natural hemin analogs are concerned (including deuteroporphyrin and mesoporphyrin derivatives), only a few compounds have been described.[19–25] Among them, the chemical structure of the bis(difluorovinyl)protoporphyrin[22] is very close to that of natural protoporphyrin. This derivative has been synthesized and tested as a compound for the photochemotherapy of cancer.[22] The use of difluorovinylporphyrins offers several advantages over other compounds in the study of hemes. First, their synthesis, performed via a Wittig reaction on pre-formed formyl derivatives, is simpler than the tedious multi-step synthesis from fluorinated pyrrole. Second, the presence of two fluorine atoms at the vinylic positions is expected not to modify dramatically the electronic or steric properties of the macrocycle. Moreover, the fluorine chemical shift sensitivity is much higher in the case of a vinylic fluorine atom than in the case of a trifluoromethyl group.[26]

In this paper, we report the synthesis of iron difluorovinyldeuteroporphyrins. The sensitivity of the fluorine chemical shifts allowed us to perform NMR kinetic measurements of the electron self-exchange transfer rate between the two redox states Fe(II) and Fe (III), using line

*Correspondence to: A. Bondon, Laboratoire de Chimie Organométallique et Biologique, UMR CNRS 6509, Université de Rennes 1, Campus de Beaulieu, 35042 Rennes Cédex, France.
E-mail: arnaud.bondon@univ-rennes1.fr

DOI: 10.1002/mrc.899

broadening analysis. Although the electron self-exchange studies could be conducted without problem in the presence of the two isomers, 2- and 4-difluorovinyldeuteroporphyrins, the absence of one vinyl side-chain could constitute a serious problem in reconstituting heme proteins. This prompted us to retain the previously reported bis(difluorovinyl)protoporphyrin.[22] Using a recent improvement in the synthesis of bis(diformyl)deuteroporphyrin,[27] the fluorinated hemin was easily prepared. In order to evaluate this fluorinated porphyrin as a ^{19}F NMR probe for heme proteins, several myoglobin complexes were prepared. Large variations in the fluorine chemical shifts were observed, not only between the different spin states of the Fe–porphyrin, but also between the oxygen and carbon monoxide adducts, demonstrating the great sensitivity of the fluorine chemical shifts to the metal environment.

Using the Marcus theory,[28] self-exchange electron transfer calculation can be performed using cross reactions between free heme and inorganic reagents. Despite the large number of heme proteins involved in electron transfer reactions, only a few studies have been carried out with model systems.[29–32] Some measurements have also been performed by Dixon and co-workers using ^1H NMR line broadening analysis.[33–36] Only one study reported the direct determination of dicyanoiron(II, III)protoporphyrin and deuteroporphyrin self-exchange electron rate constants,[34] (for a recent review, see Ref. 37), one of the limits being the complexity of the natural porphyrin proton spectra.[34]

EXPERIMENTAL

General methods

^{19}F NMR spectra were obtained, at 295 K, on a Bruker AC 300P or DRX 200 instrument equipped with a QNP probe and operating at 282 and 188 MHz, respectively. All fluorine chemical shifts were referred to external $CFCl_3$ set to 0 ppm. Proton spectra were also acquired on a Bruker DMX 500 spectrometer. UV–visible absorption spectra were recorded on a Kontron Uvikon 941 instrument. Fast atom bombardment (FAB) mass spectrometry was performed, at the CRMPO, with a Micromass ZabSpec time-of-flight (TOF) spectrometer. All solvents were distilled prior to use. Chemical reagents were obtained from commercial sources. Horse heart myoglobin was purchased from Sigma Chemical and used without further purification.

Synthesis

The difluorovinyl compounds (Fig. 1) were prepared by treatment of the corresponding formyl derivatives with triphenylphosphonium difluoromethylide, according to a slight modification of the procedure of Ando *et al*.[22] The reactions were carried out in *N*-methylpyrrolidinone,[38] which has been shown to be efficient in dissolving the porphyrin compounds.[22] The monoformyldeuteroporphyrins were prepared by formylation of deuteroporphyrin as described previously,[39] except that a deuteroporphyrin nickel complex was used instead of a copper complex. In both cases (Cu and Ni), the yields of the reaction were similar but NMR spectra are readily obtained with diamagnetic nickel complexes. 2,4-Diformyldeuteroporphyrin was obtained as reported recently.[27]

2- and 4-Difluorovinyldeuteroporphyrin (1a, 2a)

The 2- or 4-monoformyl deuteroporphyrin nickel complex (140 mg, 0.224 mol) and triphenylphosphine (1.2 g, 4.6 mmol) were carefully dried under vacuum at 70 °C for 1 h in a Schlenk tube and dissolved with 15 ml of degassed *N*-methylpyrrolidinone (NMP). To the solution warmed at 120 °C, 800 mg (5.2 mmol) of sodium chlorodifluoroacetate (previously dried under vacuum) in 15 ml of NMP were added dropwise, under argon, during the course of 15 min. After stirring for a further 30 min, the solution was poured on to ice and extracted with CH_2Cl_2. After several washes with water, the organic layer was dried with $MgSO_4$ and evaporated to dryness. Methanol was added to the solid residue to precipitate the porphyrin, the excess of PPh_3 remaining in solution. After filtration, the nickel difluorovinyldeuteroporphyrin was purified by column chromatography on silica gel with CH_2Cl_2 as the eluent (**1a** + **2a**; 101 mg, 68%). **1a**: ^{19}F NMR ($CDCl_3$), δ −82.24 [1F, t, J(F,F) = 27 Hz, J(H,F) = 27 Hz], −83.88 [1F, t, J(F,F) = 27 Hz, J(H,F) = 2 Hz]. ^1H NMR ($CDCl_3$), δ 9.53 (1 H, s), 9.50 (1 H, s), 9.45 (1 H, s), 9.33 (1 H,s), 8.69 (1 H, s), 6.34 [1 H, dd, J(H,F) = 27 Hz, J(H,F) = 2 Hz], 4.10 [4 H, t, J(H,H) = 6.5 Hz], 3.67 (6 H, s), 3.44 (3 H, s), 3.35 (3 H, s), 3.31 (3 H, s), 3.29 (3 H, s), 3.09 [4 H, t, J(H,H) = 6.5 Hz]. **2a**: ^{19}F NMR ($CDCl_3$), δ −82.29 (1F, t, J(F,F) = 27 Hz, J(H,F) = 27 Hz), −83.93 [1F, t, J(F,F) = 27 Hz, J(H,F) = 2 Hz]. ^1H NMR ($CDCl_3$), δ 9.76 (1 H, s), 9.72 (1 H, s), 9.65 (1 H, s), 9.63 (1 H,s), 8.86 (1 H, s), 6.55 [1 H, dd, J(H,F) = 27 Hz, J(H,F) = 2 Hz], 4.21 [4 H, t, J(H,H) = 6.5 Hz], 3.67 (6 H, s), 3.55 (3 H, s), 3.53 (3 H, s), 3.45 (3 H, s), 3.43 (3 H, s), 3.15 [4 H, t, J(H,H) = 6.5 Hz].

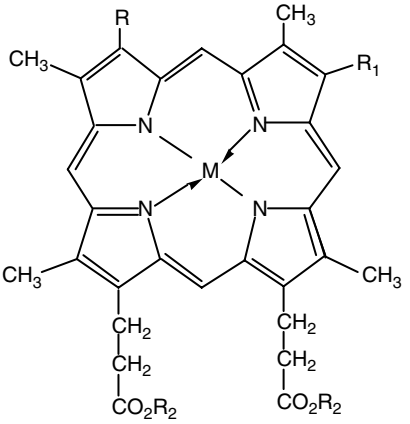

Figure 1. Structures of complexes.

Demetallation and iron insertion

The ferric complexes were obtained by classical methods.[40] Thus, removal of nickel by concentrated H_2SO_4 gave the free base porphyrins. Iron insertion was performed by heating a THF solution of the porphyrin dimethyl esters in the presence of 4 equiv. of $FeCl_2$. The porphyrin dicarboxylic acids were obtained by hydrolysis of the ester moieties in THF–KOH, then iron insertion was realized by the ferrous sulfate method leading to the complexes **1c**, **2c** and **3c**. HRMS (FAB) for **3c-Cl**: calculated, 688.1396; found, 688.1394.

Reconstitution of myoglobin with fluorinated hemins

Removal of the hemin was performed using the Teale method with ethyl methyl ketone.[41] After addition of fluorinated hemin in 0.1 M KOH to apomyoglobin, excess hemin was removed by passage through a Sephadex G-25 column. The protein was concentrated by ultrafiltration to 2×10^{-3} M in phosphate buffer (pH 7) containing 10% D_2O. Reduction was realized by addition of aqueous $Na_2S_2O_4$ in the NMR tube. Addition of cyanide in solution or bubbling of CO (or O_2) was performed directly in the NMR tube.

Sample preparation and electron transfer measurements

The fluorinated hemes (5–10 mM) were dissolved in the appropriate solvent and carefully degassed with argon. Pure 1-methylimidazole (1-MeIm) (10–20 µl) was added in the NMR tube. Reduction of the iron was performed by addition of aqueous sodium dithionite (20 mg ml^{-1}). Small aliquots (5–10 µl) were carefully introduced using a syringe because

oxidation of the metal occurred easily in the presence of air. With $CHCl_3$ some variations in the efficiency of the reducing agent were observed due to the biphasic nature of the system.

Under conditions of fast exchange in the chemical shifts time-scale, the molar fraction (f_{red}) was calculated according to the equation

$$f_{red} = \frac{\delta_{ox} - \delta_{obs}}{\delta_{ox} - \delta_{red}} \quad (1)$$

where δ_{ox} and δ_{red} are the chemical shifts of the species fully oxidized and fully reduced, respectively, and δ_{obs} is the observed chemical shift after each addition of $Na_2S_2O_4$. Electron exchange induced an increase in the linewidth of the signals of the exchanging species. The rate constant (k) is related to the total concentration of the iron porphyrin (c), to the peak widths at half-height for the exchanging, fully reduced and fully oxidized species ($W_{red,ox}$; W_{red}; W_{ox}), to the molar fractions (f_{red}, f_{ox}) and to the square of the difference, expressed in hertz, between the chemical shifts of the fluorine resonances of the pure oxidized and reduced forms ($\delta\nu$):[42]

$$k = \frac{f_{red} f_{ox} 4\pi(\delta\nu)^2}{c(W_{red,ox} - f_{red} W_{red} - f_{ox} W_{ox})} \quad (2)$$

RESULTS AND DISCUSSION

Characterization of the fluoroporphyrin iron complexes and the reconstituted myoglobin

Olefination reactions were performed following the procedure described by Ando *et al*.[22] based on a Wittig reaction of a formylporphyrin with triphenylphosphonium difluoromethylide. Initially, we used 2- and 4-monoformyldeuteroporphyrins as precursors because these compounds were easily obtained by the action of trimethyl orthoformate on deuteroporphyrin.[39] The tedious separation of these two isomers was only performed on small portions of material, permitting specific assignment of the ^{1}H and ^{19}F NMR signals of each isomer. Later, it was possible to obtain with good yields the bis(difluorovinyl)protoporphyrin using the recently reported preparation of 2,4-diformylporphyrin.[27]

The ^{19}F NMR data for difluoroporphyrin metal complexes are given in Table 1. The assignment was based on the results obtained with purified isomers and coupling constant analysis. Complexation with each of the ligands in Table 1 was checked by proton NMR spectroscopy (not shown). Large variations (45 ppm) in the fluorine chemical shifts were observed depending on the spin state of the iron. In the diamagnetic compounds, the fluorine atoms belonging to the side chains in the 2- or the 4-positions had the same chemical shifts. However, variations in the fluorine chemical shifts were observed depending on the metal ligand. For the low-spin paramagnetic complexes, the fluorine resonances were dependent on both the position of the difluorovinyl group and the nature of the axial ligand(s). With the high-spin paramagnetic compound, the linewidths of the fluorine resonances ($\Delta_{1/2} \approx 250$ Hz) prevent the observation of clear differences between the isomeric fluorine signals.

In addition to their use as model compounds, fluorinated hemin analogues were prepared to probe reconstituted heme proteins. Myoglobin is a suitable example of a heme protein readily available in various spin states and extensively characterized.[43] The great sensitivity of the fluorine chemical shifts to the spin state and redox state of the iron of the reconstituted myoglobin is demonstrated in Fig. 2.

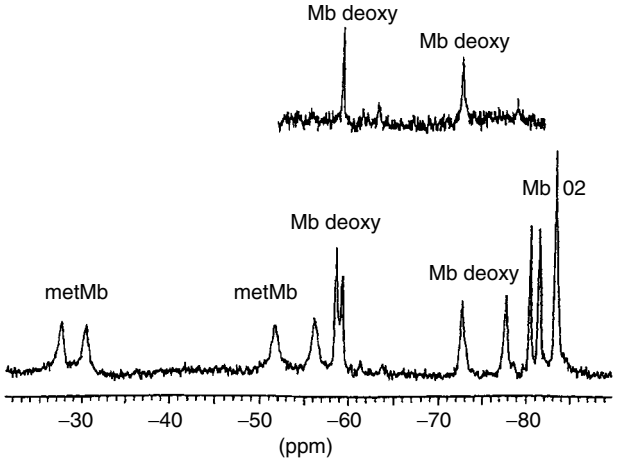

Figure 2. 188 MHz ^{19}F NMR spectrum of a mixture of three forms of myoglobin reconstituted with iron bis(difluorovinyl)protoporphyrin (**3c**). Met-, deoxy- and O_2-bound Mb signals are each labeled. The inset corresponds to the spectrum, on the same chemical shift scale, of the deoxy form of the myoglobin reconstituted with purified iron 4-difluorovinyldeuteroporphyrin (**2c**).

Table 1. Fluorine chemical shifts of 2- and 4-difluorovinyldeuteroporphyrin metal complexes[a]

M(porphyrin)	Oxidation state	Spin	4-CF_2(*trans*)	4-CF_2(*cis*)	2-CF_2(*trans*)	2-CF_2(*cis*)
FeCl	III	5/2	−42.4	−54.6	−42.4	−54.6
$Fe(Pyr)_2^+$	III	1/2	−67.7	−69.3	−68.8	−70.1
$Fe(NMeIm)_2^+$	III	1/2	−71.6	−73.9	−73.5	−76.2
$Fe(CN)_2^-$	III	1/2	−72.8	−76.2	−74.2	−77.5
$Fe(Pyr)_2$	II	0	−83.1	−86.5	−83.2	−86.6
$Fe(NMeIm)_2$	II	0	−83.5	−87.3	−83.5	−87.3
$Fe(CN)_2^{2-}$	II	0	−84.5	−87.0	−84.5	−87.0
Ni	II	0	−82.2	−83.8	−82.2	−83.8

[a] The fluorine chemical shifts were referred to external $CFCl_3$ set to 0 ppm.

Table 2. Fluorine chemical shifts of myoglobin reconstituted with iron bis(2,4)difluorovinyl)deuteroporphyrin[a]

Complex	Oxidation state	Spin	4-CF_2(*trans*)	4-CF_2(*cis*)	2-CF_2(*trans*)	2-CF_2(*cis*)
metMb(H_2O)	III	5/2	−27.1	−51.6	−29.9	−56.0
Mb(deoxy)	II	2	−58.5	−72.6	−59.2	−77.7
metMb(CN^-)	III	1/2	−71.7	−73.6	−72.7	−74.7
MbO_2	II	0	−80.3	−83.2	−81.4	−83.3
MbCO	II	0	−81.6	−84.2	−82.0	−84.4

[a] The fluorine chemical shifts were referred to external $CFCl_3$ set to 0 ppm.

The displayed ^{19}F NMR spectrum corresponds to a mixture of three different complexes of myoglobin reconstituted with iron bis(difluorovinyl)protoporphyrin (**3c**), i.e. metMb, Mb(deoxy) and Mb(O_2). The assignments were performed using the pure forms of the adducts. The chemical shifts are reported in Table 2. The specific assignment of the 2- or 4-position was based on the similarity of the chemical shifts with those observed by reconstitution of apomyoglobin with purified iron monodifluorovinyl porphyrin complexes. For example, the spectrum of Mb(deoxy) reconstituted with purified 4-difluorovinyl deuteroporphyrin iron (**2c**) is shown in the inset of Fig. 2. According to the results from the model compounds, a general upfield shift is observed for the fluorine resonances of the difluorovinyl at the 4-position versus the 2-position. All the spectra were acquired after equilibration of the heme inside the heme pocket and correspond to a single set of resonances in both ^1H and ^{19}F NMR, precluding the formation of a mixture of normal and reverse heme orientation.[43,44] A large range of fluorine chemical shifts was also observed corresponding to the different spin states and oxidation states of the myoglobin. Large variations also occur between the two types of fluorine atoms (*cis* and *trans*) for the ferric derivatives of both high- and low-spin states, whereas only weak differences are observed for the ferrous states, even in the case of the paramagnetic high-spin state. Also indicative of the high sensitivity of the fluorine resonances is the variation observed between the diamagnetic carbonmonoxy and oxy complexes. This chemical shift sensitivity of fluorine atom is expected to be larger than that of the trifluoromethyl group.[26] This is in good agreement with previously reported ^{19}F NMR data on myoglobin reconstituted with iron 3-trifluoromethylmesoporphyrin.[20] In this case, there was a range of variation, of maximum 2.3 ppm between diamagnetic ferrous and low-spin ferric complexes.[20]

Self-exchange reactions of iron fluorinated porphyrins

Metalloporphyrins are largely involved in biological electron transport systems. Whereas numerous studies have been performed on heme proteins in order to determine the factors which control the rates of electron transfer, only a few experiments have been carried out directly measuring self-exchange electron transfer between hemes.[37] However, NMR line broadening measurements have been shown to be much more reliable than measurements obtained by cross reaction between heme and inorganic reagents.[33,34]

^{19}F NMR can be used to determine the self-exchange electron transfer rate. The ferric and ferrous species are in fast exchange on the chemical shift time-scale. Consequently, very simple fluorine spectra are observed whatever the percentage of reduction. The observed chemical shift of a given fluorine resonance is the weighted average of the chemical shifts of the non-exchanging species. Ligand exchange may also contribute to the broadening of the resonances.[33,45–48] However, a ligand/porphFe ratio of >6 has been shown, even at ambient temperature, to be sufficient to suppress any contribution of ligand exchange to the line broadening of the pyrrole proton resonances.[46] Another advantage associated with ^{19}F NMR is the possibility of increasing the excess of protonated ligand. This is particularly interesting in these studies requiring a large excess of 1-MeIm.

The influence of the solvent on the electron self-exchange transfer rate of bis(1-MeIm)iron (2- and 4)-difluorovinyldeuteroporphyrins was investigated. Ferric complexes were progressively reduced by addition of small aliquots of aqueous $Na_2S_2O_4$ and ^{19}F NMR spectra were recorded for the different levels of reduction. The spectra in chloroform are displayed in Fig. 3. For each level of reduction, a single set of resonances was observed for each kind of fluorine atom. The rate constant was calculated based on line broadening analysis (Table 3), the value being independent of the level of reduction and the chosen signal, to within 10%.

Changing the nature of the solvent induced large changes in the shape of the ^{19}F NMR spectra, as shown in Figs 4 and 5. In acetone, the fluorine signals are fairly sharp, at the limit of the rate constant determination by NMR line broadening analysis, whereas in DMSO, very broad resonances are observed. The corresponding rate constants are summarized in Table 3. The variations are relatively large, from 4×10^7 mol l^{-1} s^{-1} in DMSO to 24×10^8 mol l^{-1} s^{-1} in acetone. The value of 18×10^7 mol l^{-1} s^{-1} observed in chloroform at 298 K is higher than the previously reported value of 8×10^7 mol l^{-1} s^{-1} in CD_2Cl_2 but at 252 K.[33]

According to the Marcus theory,[28] the rate constant for a self-exchange reaction can be expressed as

$$k = \kappa Z \exp(-\lambda/4RT) \quad (3)$$

where λ is the reorganization energy, κ is the probability for the electron transfer to occur and Z is the collision frequency. No work term is included in the present system because the reaction occurs between a neutral and a singly

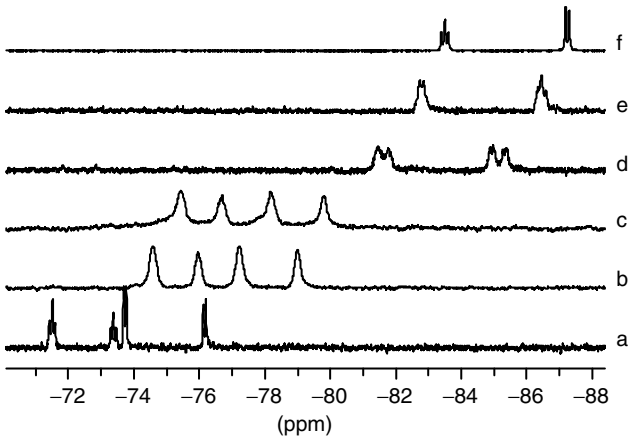

Figure 3. 282 MHz ^{19}F NMR spectra of equal amounts of bis(1-MeIm)(2- and 4-)difluorovinyldeuteroporphyrin iron complexes (**1b**, **2b**) in $CDCl_3$ at 295 K. The vertical scales are arbitrary. Various levels of reduction are displayed corresponding to serial additions of aqueous $Na_2S_2O_4$ (see Experimental section). (a) Fully oxidized; (b)–(e) partially reduced sample by successive addition of 8 μl of aqueous $Na_2S_2O_4$; (f) fully reduced corresponding to the addition of 1.3 equiv. of reducing agent. Note that whereas the two *trans* fluorines have the same chemical shift in the reduced state, they have different chemical shifts in oxidized ferric porphyrin. The same is true of the *cis* fluorines.

Table 3. Calculated self-exchange electron transfer rate constant (±10%) of bis(1-MeIm)difluorovinyldeuterohemine dimethyl ester in various solvents

Solvent	Rate constant (mol l^{-1} s^{-1})	$\frac{1}{D_o} - \frac{1}{D_s}$
Dimethyl sulfoxide	3.9×10^7	0.437
Chloroform	18.3×10^7	0.271
Acetone	24.1×10^8	0.495

charged species. Consequently, the reorganization energy is simply the sum of the inner-sphere reorganization energy, λ_{in}, and the outer-sphere reorganization energy, λ_{out}. Finally, in the case of electron transfer between ferrous and ferric iron porphyrins, λ_{in} is expected to be less than 4 kcal mol^{-1} (1 kcal = 4.184 kJ), owing to very small structural variations between the two redox states.[34] The term λ_{out}, which is related to the polarization of the surrounding environment, is defined by

$$\lambda_{out} = \left(\frac{e^2}{2r}\right)\left(\frac{1}{D_o} - \frac{1}{D_s}\right) \quad (4)$$

where e is the charge of the electron, D_o is the optical dielectric constant and D_s is the static dielectric constant. The values of $(1/D)_o - (1/D_s)$ are also presented in Table 3. For $CHCl_3$ and DMSO, the ratio of the experimental rate constants is 4.7. Using Eqns (3) and (4), the corresponding ratio can be estimated to be 5.9, which is in good agreement with the experimental data. Such an analysis of the solvent influence on the rate constants has previously been successfully

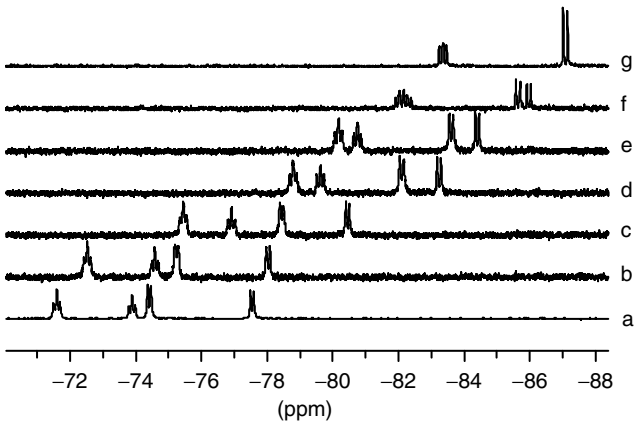

Figure 4. 282 MHz ^{19}F NMR spectra of equal amounts of bis(1-MeIm)(2- and 4-)difluorovinyldeuteroporphyrin iron complexes (**1b**, **2b**) in acetone-d_6 at 295 K. The vertical scales are arbitrary. Various levels of reduction are displayed corresponding to serial additions of aqueous $Na_2S_2O_4$. (a) Fully oxidized; (b)–(f) partially reduced sample by successive addition of 5 μl of aqueous $Na_2S_2O_4$; (g) fully reduced corresponding to the addition of 1.2 equiv. of reducing agent. Note that whereas the two *trans* fluorines have the same chemical shift in the reduced state, they have different chemical shifts in oxidized ferric porphyrin. The same is true of the *cis* fluorines.

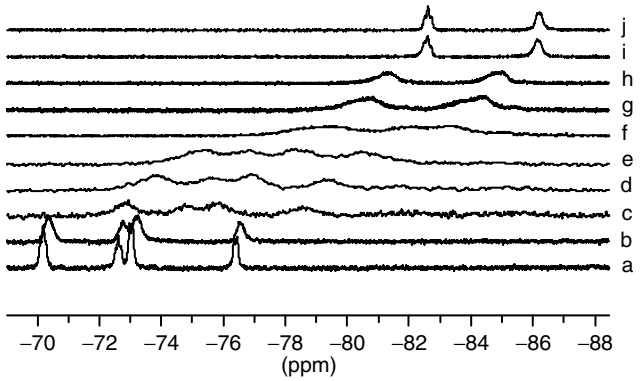

Figure 5. 282 MHz ^{19}F NMR spectra of equal amounts bis(1-MeIm)(2- and 4-)difluorovinyldeuteroporphyrin iron complexes (**1b**, **2b**) in DMSO-d_6 at 295 K. The vertical scales are arbitrary. Various levels of reduction are displayed corresponding to serial additions of aqueous $Na_2S_2O_4$. (a) Fully oxidized; (b)–(i) partially reduced sample by successive addition of 5 μl of aqueous $Na_2S_2O_4$; (j) fully reduced corresponding to the addition of 1.5 equiv. of reducing agent. Note that whereas the two *trans* fluorines have the same chemical shift in the reduced state, they have different chemical shifts in oxidized ferric porphyrin. The same is true of the *cis* fluorines.

applied to a related compound, dicyanoiron porphyrin, in DMSO and methanol.[34] However, in the case of acetone as solvent, there is no direct relation between the solvent parameters and the observed rate constant. At the present stage of investigation, there is no satisfactory explanation for this result. Extension to other solvents will be necessary for complete analysis.

CONCLUSIONS

The very high sensitivity of the fluorine chemical shifts of difluorovinyl porphyrin iron complexes is described. Dramatic simplification of the NMR spectra of reconstituted myoglobin and the structural resemblance with natural hemin make these derivatives good NMR probes for the study of heme proteins with exchangeable hemes. The simplicity of the spectra and the large chemical shift variation observed with different redox states were used to study the influence of the solvents on the self-exchange electron transfer rate constants. The measured rate constants range from 10^7 to 10^8 mol l^{-1} s^{-1}, in good agreement with previously reported values for other iron porphyrins. The behavior of the rate constants agrees well with Marcus theory for $CDCl_3$ and DMSO but not for acetone. Finally, these results clearly show that difluorovinylporphyrins are good NMR probes for large heme proteins in terms of both structural characterization and electron transfer rate analysis.

REFERENCES

1. Gerig JT. *Prog. Nucl. Magn. Reson. Spectrosc.* 1994; **26**: 293.
2. Danielson MA, Falke JJ. *Annu. Rev. Biophys. Biomol. Struct.* 1996; **25**: 163.
3. Kirk KL. In *Fluorine-containing Amino Acids: Synthesis and Properties*, Kukhar VP, Soloshonok VA (eds). John Wiley & Sons: Chichester, 1995; 343.
4. Leroy J, Cantacuzène D, Wakselman C. *Synthesis* 1982; 313.
5. Kaesler RW, Le Goff E. *J. Org. Chem.* 1982; **47**: 4779.
6. Ogoshi H, Homma M, Yokota K, Toi H, Aoyama Y. *Tetrahedron Lett.* 1983; **24**: 929.
7. Onda H, Toi H, Aoyama Y, Ogoshi H. *Tetrahedron Lett.* 1985; **26**: 4221.
8. Leroy J. *J. Fluorine Chem.* 1991; **53**: 61.
9. Barnes KD, Hu Y, Hunt DA. *Synth. Commun.* 1994; **24**: 1749.
10. Leroy J, Wakselman C. *Tetrahedron Lett.* 1994; **35**: 8605.
11. Woller EK, Smirnov VV, DiMagno SG. *J. Org. Chem.* 1998; **63**: 5706.
12. Leroy J, Bondon A, Toupet L, Rolando C. *Chem. Eur. J.* 1997; **3**: 1890.
13. Woller EK, DiMagno SG. *J. Org. Chem.* 1997; **62**: 1588.
14. Porhiel E, Bondon A, Leroy J. *Tetrahedron Lett.* 1998; **39**: 4829.
15. Porhiel E, Bondon A, Leroy J. *Eur. J. Inorg. Chem.* 2000; 1097.
16. Homma M, Aoyagi K, Aoyama Y, Ogoshi H. *Tetrahedron Lett.* 1983; **24**: 4343.
17. Aoyagi K, Haga T, Toi H, Aoyama Y, Mizutani T, Ogoshi H. *Bull. Chem. Soc. Jpn.* 1997; **70**: 937.
18. Tamiaki H, Nagata Y, Tsudzuki S. *Eur. J. Org. Chem.* 1999; 2471.
19. Billig MJ, Baker EW. *Chem. Ind. (London)* 1969; 654.
20. Toi H, Homma M, Suzuki A, Ogoshi H. *J. Chem. Soc. Chem. Commun.* 1985; 1791.
21. Snow KM, Smith KM. *J. Org. Chem.* 1989; **54**: 3270.
22. Ando A, Shinada T, Kinoshita S, Arimura N, Koyama M, Nagai T, Miki T, Kumadaki I, Sato H. *Chem. Pharm. Bull.* 1990; **38**: 2175.
23. Suzuki A, Toi H, Aoyama Y, Ogoshi H. *Heterocycles* 1992; **33**: 87.
24. Suzuki A, Tomizawa T, Hayashi T, Mizutani T, Ogoshi H. *Bull. Chem. Soc. Jpn.* 1996; **69**: 2923.
25. Shigeoka T, Kuwahara Y, Watanabe K, Sato K, Omote M, Ando A, Kumadaki I. *Chem. Pharm. Bull.* 1999; **47**: 1326.
26. Brownlee RTC, Craik DJ. *Aust. J. Chem.* 1980; **33**: 2555.
27. Kahl SB, Schaeck JJ, Koo M-S. *J. Org. Chem.* 1997; **62**: 1875.
28. Marcus RA, Sutin N. *Biochim. Biophys. Acta* 1985; **811**: 265.
29. Cassat JC, Kukururinska M, Bender JW. *Inorg. Chem.* 1977; **16**: 3371.
30. Pasternack RF, Spiro EG. *J. Am. Chem. Soc.* 1978; **100**: 968.
31. Chapman RD, Fleischer EB. *J. Am. Chem. Soc.* 1982; **104**: 1575.
32. Chapman RD, Fleischer EB. *J. Am. Chem. Soc.* 1982; **104**: 1982.
33. Dixon DW, Barbush M, Shirazi A. *J. Am. Chem. Soc.* 1984; **106**: 4638.
34. Dixon DW, Barbush M, Shirazi A. *Inorg. Chem.* 1985; **24**: 1081.
35. Shirazi A, Barbush M, Ghosh S, Dixon DW. *Inorg. Chem.* 1985; **24**: 2495.
36. Dixon DW, Woehler S, Hong X, Stolzenberg AM. *Inorg. Chem.* 1988; **27**: 3682.
37. Fukuzumi S. In *The Porphyrin Handbook*, vol. 8, Kadish KM, Smith KM, Guilard R (eds). Academic Press: San Diego, CA, 2000; 115.
38. Fuqua SA, Duncan WG, Silverstein RM. *J. Org. Chem.* 1965; **30**: 2543.
39. Monforts F-P, Scheurich G, Meier A, Haake G, Höper F. *Tetrahedron Lett.* 1991; **32**: 3477.
40. Fuhrhop J-H, Smith KM. In *Porphyrins and Metalloporphyrins*, Smith KM (ed). Elsevier: Amsterdam, 1975; 757.
41. Teale FWJ. *Biochim. Biophys. Acta* 1959; **35**: 543.
42. Chan MS, Wahl AC. *J. Phys. Chem.* 1978; **82**: 2542.
43. La Mar GN, Satterlee JD, DeRopp JS. In *The Porphyrin Handbook*, vol. 5, Kadish KM, Smith KM, Guilard R (eds). Academic Press: San Diego, CA, 2000; 185.
44. La Mar GN, Toi H, Krishnamoorthi R. *J. Am. Chem. Soc.* 1984; **106**: 6395.
45. La Mar GN, Walker FA. *J. Am. Chem. Soc.* 1972; **94**: 8607.
46. Satterlee JD, La Mar GN, Bold TJ. *J. Am. Chem. Soc.* 1977; **99**: 1088.
47. White DK, Cannon JB, Traylor TG. *J. Am. Chem. Soc.* 1979; **101**: 2443.
48. Lavalette D, Tetreau C, Momenteau M. *J. Am. Chem. Soc.* 1979; **101**: 5395.