

Handbook of Porphyrin Science

with Applications to Chemistry, Physics,
Materials Science, Engineering, Biology
and Medicine



Volume 6
NMR and EPR Techniques

Karl M. Kadish ■ Kevin M. Smith ■ Roger Guillard

Editors

 World Scientific

Contents

Preface	xiii
Contributing Authors	xv
Contents of Volumes 1–10	xxiii

29 / NMR and EPR Spectroscopy of Paramagnetic Metalloporphyrins and Heme Proteins **1**

F. Ann Walker

I. Introduction and Background	7
A. Structures and Electron Configurations of Metalloporphyrins	10
II. Principles	19
A. Proton Chemical Shifts	19
1. Contact Shifts	20
2. Pseudocontact (Dipolar) Shifts	23
a. Pseudocontact Shifts of Metalloporphyrin Substituents	23
b. Measurement of Magnetic Susceptibility Anisotropies of Ferriheme Proteins	26
c. Residual Dipolar Couplings of Proteins for Structure Determination	29
3. Temperature Dependence of Contact and Pseudocontact Shifts	31
B. Nuclear Relaxation and Linewidths	33
1. Chemical Exchange Line Broadening and EXSY Cross Peaks	33
2. Proton T_1 and T_2 Relaxation Times, as Controlled by Electron Spin Relaxation Times, T_{1e}	34
a. Electron Spin Relaxation Times, T_{1e} or τ_s	34
b. Nuclear Spin-Lattice Relaxation Times, T_1	34
c. Nuclear Spin-Spin Relaxation Times, T_2	38
C. Spin Density and Bonding: Mechanisms of Spin Delocalization	39
1. The Metal Ion	39
2. The Porphyrin Ring	40

3.	The Effect of Axial Ligand Plane Orientation on the Combined Contact and Pseudocontact Shifts of Low-Spin Ferriheme Proteins and Synthetic Hemins with Hindered Axial Ligand Rotation.	50
4.	Mechanisms of Spin Delocalization through Chemical Bonds, and Strategies for Separation of Contact and Pseudocontact Shifts	55
D.	Methods of Assignment of the ^1H NMR Spectra of Paramagnetic Metalloporphyrins	57
1.	Substitution of H by CH_3 or Other Substituent	57
2.	Deuteration of Specific Groups	58
3.	2D ^{13}C Natural Abundance HMQC Spectra and Specific or Complete ^{13}C Labeling of Protohemin for the Assignment of Heme Resonances in Proteins.	59
4.	Saturation Transfer NMR Experiments	62
5.	NOE Difference Spectroscopy	63
6.	Two-Dimensional NMR Techniques	64
III.	Spectral Analysis	69
A.	Resolution and Assignment	69
B.	Analysis of Shifts.	75
1.	Curvature in the Curie Plot over the Temperature Range of the Measurement	77
a.	Zero-Field Splitting Contributions to the Pseudocontact Shift.	79
b.	Nonzero Intercepts of the Curie Plot.	79
2.	Empirical Methods	80
3.	g -Tensor Anisotropy	83
IV.	Effect of Metal Ion and Spin State on Bonding	83
A.	Iron Porphyrins	83
1.	Iron(I) Porphyrins	83
2.	Diamagnetic Iron(II) Porphyrins	84
a.	Six-Coordinate Diamagnetic Complexes.	84
b.	Five-Coordinate Diamagnetic Complexes	85
3.	Intermediate-Spin Iron(II) Porphyrins: Observed Shifts and the Mechanism of Spin Delocalization	85
4.	Five-Coordinate High-Spin Iron(II) Porphyrins: Observed Shifts and the Mechanism of Spin Delocalization.	93
a.	Models of Deoxyhemoglobin and Deoxymyoglobin	93
b.	Models for the Reduced States of Cytochrome P450 and Chloroperoxidase	96

c.	Models for the Heme a_3 -Cu _B and Heme-Nonheme Fe Centers of Cytochrome Oxidase and NO Reductase	96
d.	Hydroxide or Fluoride Complexes	103
e.	N-Alkyl (aryl) Porphyrin Complexes	103
f.	Nitrene Complexes	104
g.	Verdoheme Analogs: Iron(II) Complexes of Octaethylxaporphyrin, OEOP	104
h.	"N-confused" or "N-inverted" Iron(II) Porphyrins and Related N-modified Macrocycle Complexes	105
5.	Possible Iron(II) Porphyrin π -Cation Radicals	106
6.	High-Spin Iron(III) Porphyrins: Observed Shifts and the Mechanism of Spin Delocalization	107
a.	Five-Coordinate, Monomeric Iron(III) Porphyrin Complexes	107
b.	Six-Coordinate Monomeric High-Spin Iron(III) Porphyrin Complexes	112
c.	Monomeric Complexes of Reduced or Oxidized Ferrihemes	117
i.	Iron(III) Sulfhemes	117
ii.	Iron(III) Octaethyl- and Tetraphenylchlorin	117
iii.	Two Iron(III) Octaethylisobacteriochlorin Isomers	121
iv.	An Iron(III) Monooxochlorin Complex	122
v.	Two Iron(III) Dioxooctaethylisobacteriochlorin Complexes	123
vi.	The Iron(III) Complex of Tetraphenyl-21-Oxaporphyrin	123
vii.	The High-Spin Iron(III) Complexes of Mono- <i>meso</i> -octaethylxaporphyrin and Mono- <i>meso</i> -octaethylazaporphyrin	124
d.	Bridged Dimeric Complexes of High-Spin Iron(III) Porphyrins and Chlorins	125
7.	Intermediate-Spin Iron(III) Porphyrins: Observed Shifts and the Mechanism of Spin Delocalization	128
8.	Low-Spin Iron(III) Porphyrins	132
a.	Griffith's Three-Orbital Theory and Experimental EPR Data for Low-Spin Iron(III) Porphyrins and Related Macrocycles	134
b.	NMR Studies of Low-Spin Iron(III) Porphyrins Having the $(d_{xy})^2(d_{xz}, d_{yz})^3$ Ground State	147

i.	Effect of Porphyrin Substituents on the Pattern of Spin Delocalization	147
ii.	The Shifts of Coordinated Imidazole Ligands and the Effect of Imidazole Deprotonation on the Pattern and Extent of Spin Delocalization	151
(a)	Neutral Imidazole Ligands	151
(b)	Imidazolate Ligands	152
iii.	Effect of Imidazole Plane Orientation on the Paramagnetic Shifts of Low-Spin Iron(III) Porphyrins	152
iv.	Bis-Ammine, Amino Ester and Phosphine Complexes	159
v.	Mixed-Ligand Complexes	160
c.	Observed Shifts and the Mechanism of Spin Delocalization for the $(d_{xz}, d_{yz})^4(d_{xy})^1$ Ground State	161
d.	The Mixed Ground State Behavior of Bis-Cyanide Complexes of Low-Spin Ferrihemes: Observed Shifts and the Mechanism of Spin Delocalization	164
e.	The Mixed Ground State Behavior of Bis-(pyridine) Complexes of Low-Spin Ferrihemes: Observed Shift Trends and the Mechanism of Spin Delocalization	167
f.	The Mixed Ground State Behavior of Bis-(pyridine) Complexes of Low-Spin Iron(III) Complexes of Oxophlorins and <i>Meso</i> -Amino Porphyrins	174
g.	Low-Spin Fe ^{III} Complexes of <i>Meso-Meso</i> -Linked 5,5'-Bis(10,15,20-Triphenylporphyrin)	178
h.	Five-Coordinate Low-Spin Iron(III) Porphyrins and a Porphycene	179
i.	Low-Spin Iron(III) Complexes of Reduced Hemes	182
j.	Low-Spin Iron(III) Complexes of N-Alkylporphyrins	186
k.	Thermodynamics of Axial Ligation of Iron(III) Porphyrins	186
l.	Kinetics of Axial Ligand Exchange	187
9.	Electron Exchange Between Low-Spin Iron(III) and Low-Spin Iron(II) Porphyrins	188
10.	¹ H and ¹³ C NMR Spectroscopy of High- and Low-Spin Ferriheme Proteins: The Nitrophorins and Heme Oxygenases	190
a.	NMR Spectroscopy of the Nitrophorins	190

i.	NMR Investigations of the High-Spin Forms of the Nitrophorins from <i>Rhodnius prolixus</i>	196
ii.	pH Titration of the High-Spin Nitrophorins from <i>Rhodnius prolixus</i>	204
iii.	NMR Investigations of the Low-Spin Forms of the Nitrophorins from <i>Rhodnius prolixus</i> , and Comparison to Other Heme Proteins	207
iv.	Heme Ruffling of the Nitrophorins and Comparison to Other Heme Proteins	217
v.	Nitrite Reductase Activity of Nitrophorin 7	221
vi.	Dimerization of NP4	222
vii.	NMR Spectroscopy of Apo-Nitrophorin 2	223
b.	NMR Spectroscopy of the Hemin-Containing Heme Oxygenases	225
i.	NMR Study of High- and Low-Spin Mammalian Heme Oxygenases	227
ii.	NMR Studies of Bacterial Heme Oxygenases	230
(a)	Heme Propionate-Polypeptide Interactions Dictate Regioselectivity in HOs	231
(b)	NMR Studies of Heme Electronic Structure and its Potential Implications to the Mechanism of Heme Oxidation	236
(c)	NMR Spectroscopic Studies of Dynamic Reactivity Relationships	243
c.	NMR Spectroscopy of Miscellaneous Other Heme Proteins	249
11.	Iron(III) Macrocycle π -Cation Radicals	251
a.	High-Spin Iron(III) Porphyrin π -Cation Radicals	251
b.	Spin-Admixed and Intermediate-Spin Iron(III) Porphyrin π -Cation Radicals	253
c.	Low-Spin Iron(III) Porphyrin π -Cation Radicals	254
d.	Iron(III) π -Cation Radicals of Oxophlorins	254
e.	Iron(III) Corrole π -Radicals	256
12.	Iron(IV) Porphyrins	258
a.	Six-Coordinate, Bis-Methoxide Iron(IV) Porphyrins	259
b.	Five- and Six-Coordinate Ferryl, (Fe=O) ²⁺ , Porphyrin Complexes	259
c.	Five-Coordinate Iron(IV) Phenyl Porphyrins	260
d.	Comparison of Iron(IV) Porphyrins and Iron(III) Porphyrin π -Radicals	261

13. Iron(IV) Porphyrin π -Radicals	261
14. Iron(V) Porphyrins	266
B. Ruthenium and Osmium Porphyrins	266
C. Cobalt Porphyrins	268
1. Low-Spin Cobalt(II) Porphyrins	268
a. Observed Shifts and the Pseudocontact Interaction	268
b. Oxidation of Cobalt(II) Porphyrins to Produce π -Radical Dimers	270
c. Low-Spin Cobalt(II) Oxaporphyrins and Porphodimethenes	271
d. High-Spin Cobalt(II) N-Alkylporphyrins and Alkoxy Adducts of Oxaporphyrins	272
2. High-Spin Cobalt(II) Complexes of a Weak-Field Porphyrin Ligand	273
3. Alkylcobalt(III) Porphyrins: Agostic Interactions or Paramagnetic Excited States?	273
4. A Cobalt(III) Porphyrin π -Cation Radical	275
D. Rhodium Porphyrins	275
E. Manganese Porphyrins	278
1. High-Spin Manganese(II) Porphyrins	279
2. High-Spin Manganese(III) Porphyrins	279
3. Low-Spin Manganese(III) Porphyrins	280
4. "Manganese(III) Corrole" at Low Temperatures = Manganese(II) Corrole π -Cation Radical at Ambient Temperatures	280
5. Manganese(III) Porphyrin π -Radicals and Their Transformation to Dichloromanganese(IV) Porphyrins	283
6. Oxomanganese(IV) Porphyrins	284
F. Nickel Porphyrins	284
G. Lanthanide Porphyrins	292
H. Miscellaneous Metalloporphyrins which Have Extremely Broad Lines	294
1. Copper(II) and Silver(II) Porphyrins	294
2. Vanadium(IV) Porphyrins	297
3. Chromium Porphyrins	298
I. Summary of Paramagnetic Shifts and Mechanisms of Spin Delocalization for the Metalloporphyrins	299
V. Acknowledgments	303
VI. References	304

30 / Heme Acquisition by Hemophores: A Lesson from NMR 339

Paola Turano

List of Abbreviations	340
I. Biological Background	340
II. Hemophore Protein HasA	342
A. Heme-Loaded HasA	342
1. NMR of the Gallium(III) Derivative	344
2. NMR of the Iron(III) Derivative	344
a. ¹ H NMR	344
b. Heteronuclear Detection	346
i. ¹³ C NMR	346
ii. ¹⁵ N NMR	349
B. The H83A Variant	350
C. Apo HasA	353
III. HasA–HasR Interaction	353
A. ¹ H– ¹⁵ N NMR Spectra	353
1. Chemical Shift Perturbation Mapping	356
2. Spectral Profiling	357
B. The Fate of the Heme	358
IV. Interaction with Hemoglobin	359
V. Concluding Remarks	360
VI. Acknowledgments	361
VII. References	361

31 / Structure–Function Relationships Among Heme Peroxidases: New Insights from Electronic Absorption, Resonance Raman and Multifrequency Electron Paramagnetic Resonance Spectroscopies 367

Giulietta Smulevich, Alessandro Feis, Barry D. Howes and Anabella Ivancich

I. General Introduction	368
A. Resonance Raman Spectroscopy	370
B. Multifrequency Electron Paramagnetic Resonance Spectroscopy	370
II. Superfamily of Plant, Fungal, and Bacterial Peroxidases	372
A. Heme Pocket	373
1. Fe(III) Resting State	374
2. Extended Network of H-Bonds	380

3. Vinyl-Protein Interaction	381
4. Imidazolate Character of the Proximal Iron Ligand.	384
B. Heme Pocket in Catalase-Peroxidases	386
1. KatG from <i>Synechocystis</i>	390
2. KatG from <i>Mycobacterium tuberculosis</i>	394
C. Calcium Binding Sites	396
D. Binding Sites for Substrates: Benzohydroxamic and Salicylhydroxamic Acids	400
E. Ligand Binding	403
F. Catalytic Intermediates	410
1. X-Ray Structures of Intermediates	412
2. Resonance Raman Characterization of Intermediates	416
3. Multifrequency EPR Spectroscopy: Identification and Reactivity of Intermediates	422
III. Superfamily of Animal Peroxidases	429
A. Covalent Links and Heme Structure	430
B. X-Ray Structures: An Overall View	431
C. Resonance Raman and Electronic Absorption Spectroscopies . .	432
D. Catalytic Intermediates	436
1. Resonance Raman and Electronic Absorption Studies.	436
2. Multifrequency EPR Spectroscopy Combined with Stopped-Flow Electronic Absorption Spectrophotometry. . . .	438
IV. Acknowledgments	442
V. References.	442
Index.	455