

Biological Magnetic Resonance
Volume 14

Spin Labeling
The Next Millennium

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Biological Magnetic Resonance
Volume 14

Spin Labeling
The Next Millennium

Edited by

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Columbus, Ohio

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To Harden M. McConnell and Eduard G. Rozantsev

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Preface

We present here the second issue devoted entirely to the spin-labeling technique as part of *Biological Magnetic Resonance*. Volume 14 commemorates a modification in our editorial policy with the retirement of my esteemed coeditor, Jacques Reuben. From this juncture into the future, each issue will focus on some special topic in magnetic resonance. Each volume will be organized in most cases by guest editors, for example forthcoming issues will address the following topics:

¹³C *in vivo* magnetic resonance (P. Robitaille and L. J. Berliner, eds.)

Modern techniques in proton NMR of proteins (R. Krishna and L. J. Berliner, eds.)

Instrumental techniques of EPR (C. Bender and L. J. Berliner, eds.)

The current volume, *Spin Labeling: The Next Millennium*, presents an excellent collection of techniques and applications that evolved during the past decade since the last volume, volume 8 (1989). Some obvious omissions, such as multi-quantum EPR and very high-frequency FT-ESR were unfortunately not possible for this volume. Perhaps they will appear in *Spin Labeling: 2001*.

Lastly it is a pleasure to honor two scientists whose contributions were both pioneering and pivotal to the spin label technique: Professor Eduard G. Rozantsev (Moscow), whose synthetic feats in nitroxyl chemistry set the broad stage for a versatile catalog of labels; and Professor Harden M. McConnell, last year's International ESR (EPR) Society Gold Medalist, who conceived and developed the spin label technique to address many biological problems (proteins, enzymes, membranes, cells, immune response, etc.).

Lawrence J. Berliner
Columbus, Ohio

Contents

Introduction: Reflections on the Beginning of the Spin Labeling Technique

Lawrence J. Berliner

Chapter 1

Analysis of Spin Label Line Shapes with Novel Inhomogeneous Broadening from Different Component Widths: Application to Spatially Disconnected Domains in Membranes

M. B. Sankaram and Derek Marsh

1.	Introduction	5
1.1.	Inhomogeneous Broadening Due to Unresolved Hyperfine Structure	6
1.2.	Inhomogeneous Broadening Due to Different Component Widths	8
2.	Theory	8
2.1.	Sum of Two Spectra with Different Widths	9
2.2.	Statistical Distribution of Spin Labels	11
2.3.	Methods to Determine the Existence of a Statistical Distribution of Spin Labels	13
2.4.	Experimental Considerations	16
3.	Applications	16
3.1.	Domain Structure in a Two-Component, Two-Phase Lipid Bilayer System	16
3.2.	Effect of a Peptide on Domain Structure	21
4.	Conclusions	21
	<i>References</i>	21

Chapter 2

Progressive Saturation and Saturation Transfer EPR for Measuring Exchange Processes and Proximity Relations in Membranes*Derek Marsh, Tibor Páli, and László I. Horváth*

1. Introduction	23
1.1. Continuous Wave Saturation	25
1.2. Effective Spin Lattice Relaxation Times	26
2. Contributions to Intrinsic Spin-Lattice Relaxation Rates	28
2.1. Electron Relaxation Mechanisms	28
2.2. Nuclear Relaxation Mechanisms	35
3. Paramagnetic Relaxation Enhancement	37
3.1. Paramagnetic Enhancement by Heisenberg Exchange	38
3.2. Paramagnetic Enhancement by Magnetic Dipole-Dipole Interactions	40
4. Spin Lattice Relaxation Enhancement by Exchange Processes	42
4.1. Two-Site Exchange	44
4.2. Heisenberg Spin Exchange	47
5. Continuous Wave Saturation Measurements	52
5.1. Progressive Saturation EPR	52
5.2. Saturation Transfer EPR	56
6. Applications	58
6.1. Two-Site Lipid Exchange and Lipid-Protein Interactions	59
6.2. Lipid and Protein Collision Frequencies: Translational Diffusion	63
6.3. Spin Label Location and Accessibility: Paramagnetic Relaxation	69
6.4. Distance Measurements: Dipolar Relaxation	72
6.5. Slow Rotational Diffusion and Saturation Studies	77
7. Conclusions	79
<i>References</i>	80

Chapter 3

Comparative Spin Label Spectra at X-Band and W-band*Alex I. Smirnov, R. L. Belford, and R. B. Clarkson*

1. Introduction	83
2. Comparative X- and W-Band EPR Spectra of Spin Labels in Isotropic Liquids	84
2.1. Solution of Perdeuterated Tempone in Toluene	85

2.2.	Solution of 3-Doxyl-17 β -Hydroxy-5 α -Androstane in <i>O</i> -xylene	88
2.3.	Resolution Enhancement in High Magnetic Fields: Mixtures of Phenyl- <i>tert</i> -butylnitron Spin Adducts	90
3.	Sample Deoxygenation in CW HF EPR Experiments	93
4.	Comparative X- and W-Band EPR Spectra of Spin-Labeled Phospholipids and Proteins	95
4.1.	Small Spin Labels in Phospholipid Membranes	95
4.2.	Doxyl Stearic Acid Labels in Phospholipid Membranes	97
4.3.	Binding Spin-Labeled Fatty Acids to Bovine Serum Albumin	100
4.4.	Spin-Labeled Proteins: Immobilized Labels	102
4.5.	Spin-Labeled Proteins: Mobile Labels	104
	<i>References</i>	106

Chapter 4

Use of Imidazoline Nitroxides in Studies of Chemical Reactions: ESR Measurements of the Concentration and Reactivity of Protons, Thiols, and Nitric Oxide

Valery V. Khrantsov and Leonid B. Volodarsky

1.	Introduction	109
2.	Stable Nitroxides in Proton Exchange Reactions	110
2.1.	Theoretical Considerations	110
2.2.	Effect of pH on ESR Spectra of Stable Nitroxides	112
2.3.	pH-Sensitive Nitroxides in Studies of Proton Exchange Reactions	125
2.4.	ESR Measurements of the Local Concentration of Protons	130
2.5.	ESR Studies of Proton-Related Transport Processes	135
2.6.	pH-Sensitive Spin Labels in Studies of Peptides, Proteins, and Biomembranes	140
3.	Disulfide Biradicals in the Thiol-Disulfide Exchange	149
3.1.	Physicochemical Backgrounds	149
3.2.	Quantitatively Determining Thiol Groups in Biological Systems	155
3.3.	ESR Studies of Thiol-Related Processes	157
3.4.	Spin Labeling SH Groups in Proteins	159
4.	Reactions of Nitronyl Nitroxides with Nitric Oxide	161
4.1.	Trapping Nitric Oxide by Nitronyl Nitroxides: Physicochemical Studies	162

4.2. ESR Detection of Nitric Oxide in Chemical and Biological Systems	168
4.3. Antagonistic Action of NNR against Nitric Oxide	172
5. Conclusions	174
<i>References</i>	176

Chapter 5

ENDOR of Spin Labels for Structure Determination: From Small Molecules to Enzyme Reaction Intermediates

Marvin W. Makinen, Devkumar Mustafi, and Seppo Kasa

1. Introduction	181
2. EPR and ENDOR Spectroscopy of Nitroxyl Spin Labels	183
2.1. EPR of Nitroxyl Spin Labels	183
2.2. Basis of ENDOR	187
2.3. ENDOR of Nitroxyl Spin Labels	190
2.4. TRIPLE Spectroscopy of Nitroxyl Spin Labels	201
3. X-ray Structure and Conformation of Nitroxyl Spin Labels	203
3.1. Molecular Structure of Nitroxyl Spin Labels	203
3.2. Molecular Modeling of Nitroxyl Spin Labels	206
4. Structure and Conformation of Spin-Labeled Molecules by ENDOR	211
4.1. Spin-Labeled Amino Acids	211
4.2. Other Spin Label Derivatives	225
5. Intermediates of Enzyme-Catalyzed Reactions	229
5.1. Methods of Stabilizing Reaction Intermediates	229
5.2. Structural Characterization of True Intermediates of Enzyme-Catalyzed Reactions by ENDOR	231
6. Future Directions	240
6.1. Enhanced Resolution of ENDOR Spectra	240
6.2. Enzyme Reaction Intermediates	242
6.3. Spin-Labeled Proteins for ENDOR	244
<i>References</i>	245

Chapter 6

Site-Directed Spin Labeling of Membrane Proteins and Peptide-Membrane Interactions

Jimmy B. Feix and Candice S. Klug

1. Introduction	251
---------------------------	-----

2.	The SDSL Paradigm	252
2.1.	Spin Labeling Cysteine	253
2.2.	Spin Label Motion in SDSL	253
2.3.	CW Saturation in SDSL	255
3.	SDSL of Membrane Proteins	259
3.1.	Structure–Function Studies of α - Helical Membrane Proteins	259
3.2.	SDSL of the Ferric Enterobactin Receptor FepA	265
3.3.	Metal-Nitroxide Distance Measurements	271
4.	SDSL Studies of Peptide-Membrane Interactions	273
4.1.	Diphtheria Toxin	273
4.2.	Cecropins	274
4.3.	Alamethicin	276
4.4.	Influenza Virus Hemagglutinin	276
4.5.	Melittin	277
5.	Conclusion	278
	<i>References</i>	279

Chapter 7

Spin-Labeled Nucleic Acids

Robert S. Keyes and Albert M. Bobst

1.	Introduction	283
2.	Syntheses of Spin-Labeled Nucleic Acids	288
2.1.	Chemical Oligonucleotide Synthesis	288
2.2.	Enzymatic Incorporation of Spin-Labeled Substrates	292
2.3.	Spin Labeling Oligonucleotides by Spin Trapping	295
3.	Characterization of Spin-Labeled Nucleic Acids	297
3.1.	Free Spin Test	297
3.2.	EPR-Specific Activity of Spin-Labeled Nucleic Acids	298
3.3.	Influence of Spin Label on Native Nucleic Acid Duplex Conformation	301
3.4.	Evaluation of Spin-Label Rigid-Limit Tensors	302
4.	Motional Analysis of Spin-Labeled Nucleic Acids	304
4.1.	Theoretical Approaches to Modeling Nucleic Acid Dynamics	304
4.2.	Spin-Labeled Nucleic Acid Dynamics Detected by EPR	306
5.	Biological Applications of Spin-Labeled Nucleic Acids	319
5.1.	Spin-Labeled Nucleic Acids As Monitors in Biological Studies	319
5.2.	Spin-Labeled Hybridization Probes	320
5.3.	Detecting Local Z-DNA Conformations	323

5.4. Detecting Local DNA Bending	325
5.5. Relative Binding Affinities of Single-Strand Nucleic Acid Binding Proteins	327
5.6. Monitoring the Dynamics of <i>EcoRI</i> Endonuclease and Homeodomain Protein Spin-Labeled Oligonucleotide Complexes	329
6. Conclusions	334
<i>References</i>	334

Chapter 8

Spin Label Applications to Food Science

Marcus A. Hemminga and Ivon J. van den Dries

1. Introduction	339
2. Spin Labels	340
3. Analysis of ESR and ST-ESR Spectra	341
4. Molecular Motion in Liquid Samples	344
4.1. Glycerol Water Systems	345
4.2. Sucrose Water Systems	347
5. Molecular Mobility in Sugar Water Glasses	348
5.1. Glassy State	349
5.2. Sugar Water Systems	349
5.3. Molecular Mobility in Maltooligosaccharide Water Glasses	351
5.4. Glucose Water Systems	354
5.5. Commercial Maltodextrin	355
5.6. Activation Energy for Molecular Reorientation	356
6. Gluten Systems	356
6.1. ESR Spectra	357
6.2. ST-ESR Spectra	358
7. Conclusions	359
Appendix: Calibration Procedure for ST-ESR Spectra	361
Calibration of ST-ESR Spectra	361
Notes	365
<i>References</i>	365

Chapter 9

EPR Studies of Living Animals and Related Model Systems (*In Vivo* EPR)

Harold M. Swartz and Howard Halpern

1. Introduction	367
---------------------------	-----

2. Rationale for Developing and Using <i>in Vivo</i> EPR	368
3. Imaging and Spectroscopy	370
4. Potential Constraints on <i>in Vivo</i> EPR	371
4.1. Nonresonant Absorption of the Exciting Frequency (Usually Microwaves)	372
4.2. Concentration of Paramagnetic Species	372
4.3. Bioreduction	373
4.4. Excretion	374
4.5. Physiological Motions	374
4.6. Accurate Localization of Paramagnetic Materials	374
4.7. Penetration of Microwaves	374
5. Sensitivity Considerations for <i>in Vivo</i> EPR	375
5.1. Concentrations of Naturally Occurring Free Radicals and Other Paramagnetic Materials <i>in Vivo</i>	375
5.2. Sensitivity of <i>in Vivo</i> EPR Spectrometers	375
6. Results of <i>in Vivo</i> EPR Spectroscopy of Functional Biological Systems	376
6.1. Initial Studies	376
6.2. Types of Studies with <i>in Vivo</i> EPR Spectroscopy	377
6.3. Specific Results Obtained with <i>in Vivo</i> EPR Spectroscopy	378
7. Results of <i>in Vivo</i> EPR Imaging of Functional Biological Systems	390
7.1. Imaging Studies of Pharmacokinetics of Nitroxides	391
7.2. Imaging Studies of Tumors and Tumor Oxygenation	393
7.3. Imaging of Diffusion in Skin and Related Systems	393
7.4. Imaging Studies of the Concentration of Nitric Oxide	394
7.5. Imaging Studies at the Microscopic Level	394
7.6. Use of Gated and Pulsed Methods to Obtaining Images	394
7.7. Using of Pulsed Methods in <i>In Vivo</i> EPR	395
7.8. Review of Spectral Spatial-Imaging Studies	395
7.9. Issues in EPR Imaging	396
8. Summary and Conclusions	397
References	397

Appendix

Derek Marsh and Karl Schorn

References	409
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Contents of Previous Volume in Series	411
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Index	415
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Biological Magnetic Resonance

Volume 14

Spin Labeling

The Next Millennium

Introduction

Reflections on the Beginning of the Spin Labeling Technique

Lawrence J. Berliner

This volume is dedicated to the two pioneers who contributed seminally to the beginnings of the spin label technique, Eduard G. Rozantsev, who developed a broad range of nitroxyl compounds and reagents; and Harden M. McConnell, whose foresight into the areas of biological structure and function brought the world the broad leadership that guided this technique to where it is today.

As Harden McConnell's first graduate student assigned to spin labeling, I take great pride in reflecting on the early days and subsequent development of the technique from the early- to mid-60s when it first began. McConnell's lab was located in the Physical Chemistry Laboratories of the Department of Chemistry at Stanford University, where his research to that point had been heavily involved in areas of chemical physics, solid-state physics, and theoretical aspects of spin physics. Rozantsev's lab, then at the Institute of Chemical Physics, USSR Academy of Sciences, Moscow, was publishing extensively on the chemistry, synthesis and physical chemistry of several nitroxyl compounds (coined iminoxyl at the time); this research was published mainly in Russian academy journals. Since it was difficult to obtain samples from Moscow at that time, a major synthetic effort was launched in the McConnell lab to reproduce compounds synthesized in the Rozantsev

lab and to develop analogues of protein modification reagents. In fact early during this period, the first *in vivo* related experiment was done in the McConnell lab to assess toxicity of nitroxyl compounds to cells or living systems. Several grams of di-*t*-butyl nitroxide were dissolved in an aquarium where a goldfish swam. The goldfish survived the nitroxyl environment, but when someone allowed hot water to drip slowly into the aquarium, the animal died either from heat exposure or by falling out of the aquarium when the water level reached the top and overflowed into the sink! Another effort involved analyzing EPR lineshape and motional characteristics of the tumbling nitroxyl group. The first paper (Stone *et al.*, 1965) reflected the effort of three postdoctorates, two of whom were trained as organic chemists; the latter two authors (including McConnell) were experts in lineshape analysis. The major efforts in the McConnell laboratory from 1966–67 focused predominantly on protein and enzyme problems. There was another massive effort by a team of postdoctorates who attempted to apply, in a blanket fashion, a new maleimide spin label to a variety of enzymes and proteins (Griffith and McConnell, 1966).

The common attributes of McConnell and Rozantsev is that they were great scientists, interested purely in the joy of pursuing important fundamental problems (instead of what was currently politically in favor.) To the best of my knowledge, McConnell's conception of the spin-labeling technique was based heavily on the pioneering fluorescence work of Gregorio Weber (University of Illinois), who studied fluorescence depolarization, and other steady-state techniques with extrinsic fluorophores introduced into specific sites in proteins and enzymes. At the same time Stanford hired Lubert Stryer in the medical school (Department of Biochemistry), who was a consultant in protein structure/function. Nonetheless, it is fair to say that most of the driving force, motivation, and discovery came from within the McConnell group through the foresight and leadership of its mentor. This of course led to the applications of spin labeling in several other areas of biochemistry and medicine: lipids and membranes, cellular machinery (such as the complement system), studies of diffusion phenomena within lipid bilayers. Most recently there were impressive *in vivo* applications in animals, as outlined in the chapter by Swartz and Halpern. McConnell also applied nitroxyl groups as paramagnetic relaxation agents to probe protein structure by NMR. This was elegantly described in volume 8 of this series by Anglister (in Berliner and Reuben, 1989). McConnell was literally the father, grandfather, and great-grandfather of spin labeling. Rarely a publication on the subject does not cite at least one or two of his fundamental papers. It is certainly an honor and a tribute to recognize his contributions in this book.

Eduard Rozantsev was a prolific chemist who was also well-versed in physical chemistry and biochemistry as well. Under the tutelage of M. B. Neiman at the Institute of Chemical Physics, he grew to be an international giant in this field, collaborating frequently with other physical and biophysical chemists at the Institute on applications of nitroxyl compounds, some modeled after McConnell's early articles. Unfortunately during Soviet times the Institute of Chemical Physics was

not always headed by extremely benevolent directors. For reasons (all of which are not totally known to me) Rozantsev fell into disfavor with Director V. Buchachenko, who banished him from the Institute in the later 1970s. Rozantsev took a position in the Institute for Applied Biotechnology in Moscow, where he continued a research and teaching program, albeit much smaller than that at the Institute of Chemical Physics. During the time period of Buchachenko's reign, Rozantsev was frequently denied travel; for example he did not attend the First International Symposium on Nitroxyl Compounds in Pečs, Hungary nor the Second International Symposium in Novosibirsk in 1989. Nonetheless this editor had the pleasure and honor of meeting him twice in Moscow, once in 1981 on a National Academy of Sciences exchange visit to the Shemyakin Institute of Organic Chemistry, where we were allowed to have a private meeting. This was shortly after Rozantsev and Renat Zhdanov undertook the massive effort of translating the first volume of *Spin Labeling: Theory and Applications* (Berliner, 1976) into Russian (Rozantsev, 1979) for Mir Press. The second visit was in 1994 when I met him and one of his postdoctoral students (an excellent physical chemist who carried on the tradition of high-quality organic synthesis, characterization, and new insights into novel structures as a result of Rozantsev's excellent mentoring). Rozantsev was also a true giant in his field. His book, *Free Nitroxyl Radicals* (Rozantsev, 1970) still serves as a useful guide. In fact, it is noteworthy that we continue the tradition in every spin-labeling volume of including a complete chapter on new aspects of organic synthesis of nitroxyl compounds, each containing detailed methods and materials sections enabling a chemist to carry out synthesis without referring to the original publication.

The spin-labeling field blossoms even more as we head into the next millennium. As exemplified by the chapters here, it has been applied to systems in food chemistry, nucleic acid and nucleotide biochemistry, to cell applications, *in vivo* applications with small animals, and most recently as a tool for specifically incorporating spin labels at unique amino acid residue positions in mutated proteins. As described in other chapters, new instrumental advances combined with other techniques, such as NMR (ENDOR), allow us to probe detailed motional and structural characteristics, especially at very high frequencies (e.g., 95 GHz). No doubt there are many other techniques yet to come. In conclusion, speaking for the other authors as well, we hope that the subject matter in this volume and continuing contributions from the two honored scientists and their research groups inspire into even more applications and techniques in the millennium to come!

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