viation between first-order predicted and simulated cross-peak ratios is due to this effect at a mixing time of 50 ms.

It is difficult to generalize the above results to assess the magnitude of error that would be introduced in internuclear distance calculations based on first-order theory because of the many different spin systems, motional time scales, and internuclear distance combinations encountered in NOESY spectra of proteins. We can, however, provide some crude guidelines for analysis of cross-peaks involving geminal proton pairs in moderately sized proteins. From the above discussion it is clear that secondary through-space transfers are capable of producing large errors in distances calculated between either member of the geminal pair and a neighboring proton. For example, simulation of cross-peak intensities of glycine residue 33 in ACP in the limiting case where $J_{AB}/\delta = 0$ (see Table I) shows that first-order interpretation would lead to an error in the NH-α-CH distance ratio of 8.5% for a mixing time of 150 ms. This error can be reduced to 4.5% by using a mixing time of 50 ms. When strong scalar coupling exists, first-order interpretation of the data can also produce distance errors. The effects due to strong J coupling, however, tend to be obscured by secondary through-space transfers except at short mixing times and for very strongly coupled spins ($J/\delta = 0.5$). These effects are probably not of great consequence since in general it is not possible to integrate accurately cross-peaks that arise from very strongly coupled spins. Thus, first-order theory is appropriate for most J/δ ratios for which resolvable cross-peaks arise but interpretation of distances from cross-peaks involving geminal proton pairs should be relegated to data sets collected with short mixing times.

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Determination of Vinyl Orientation in Resting State and Compound I of Horseradish Peroxidase by the ^{1}H Nuclear Overhauser Effect

V. Thanabal,[1a] Jeffrey S. de Ropp,[1b] and Gerd N. La Mar*[1a]

Department of Chemistry and UCD NMR Facility
University of California, Davis, California 95616
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There exists a large body of indirect evidence that the heme pocket of horseradish peroxidase, HRP, is stereochemically more rigid and buried than in myoglobin or hemoglobin.[2-6] One of the manifestations of this influence is the clamping of the heme vinyls so as to restrict their oscillatory mobility and force a more in-plane orientation than in other hemoproteins.[3-7] Such in-plane orientations have been indirectly supported by both ^{1}H NMR[3-5] and resonance Raman,[6,7] RR, spectral interpretations and rationalized to enhance the stability of the doubly oxidized reactive intermediate, compound I, HRP-I. The inability to grow adequate single crystals, however, has prevented the usual confirmation of such structural details by X-ray diffraction. It would be useful to have not only more direct evidence for such in-plane orientations but also to distinguish among two types of in-plane orientations, *cis* or *trans* (as depicted for the 4-vinyl and 2-vinyl groups, respectively, in F of Figure 1). RR studies on reduced HRP have indicated *cis* orientations for both vinyls.[6]

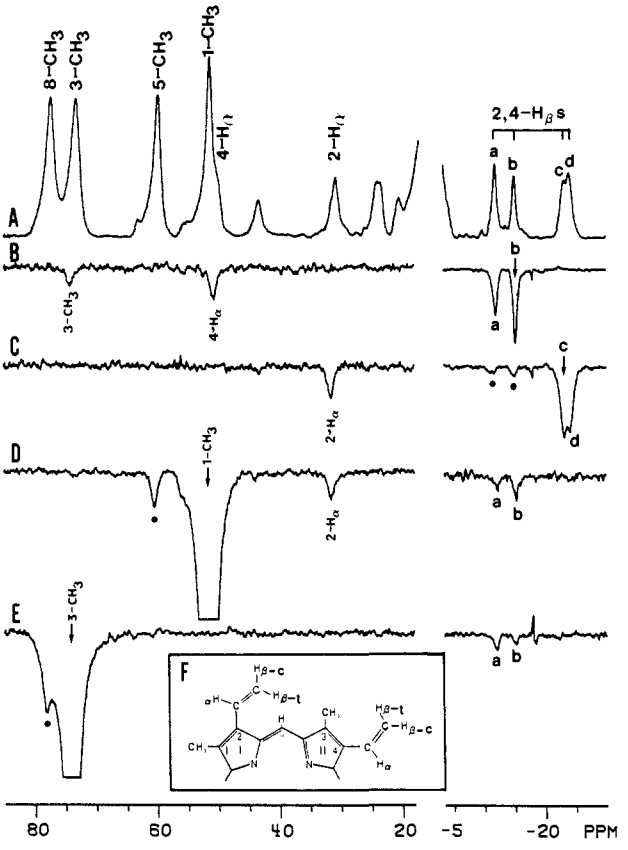

Figure 1. 360-MHz ^{1}H NMR spectrum of (A) 3 mM HRP-I in ^2H_2O at 15 °C, pH 7.0. Previously assigned resonances[4] are labeled. (B)-(E) are the NOE difference spectra generated by subtracting the reference spectrum with the decoupler off-resonance from a similar spectrum of the same sample in which the desired resonance was saturated for 30 ms with a 50-mW decoupler pulse. Spectra were collected by the Redfield 21412 pulse sequence. The upfield portions were recorded with the carrier at -18 ppm while the downfield portions were collected with the carrier centered at 55 ppm. In each of the difference spectra (B)-(E), an arrow indicates the peak being saturated. A filled circle denotes off-resonance power spillage. (B) Saturate b; note NOEs to a and 4-H$_\alpha$. (C) Saturate c; note NOE to 2-H$_\alpha$. (D) Saturate 1-CH$_3$; note NOE to 2-H$_\alpha$; the NOEs to a and b are due to the partial saturation of the overlapping 4-H$_\alpha$ peak. (E) Saturate 3-CH$_3$; note NOEs to peaks a and b. (F) Portion of hemin possessing the two vinyl groups; the 2-vinyl is depicted in the *trans* and the 4-vinyl in the *cis* orientations.

In principle, the homonuclear Overhauser effect,[8] NOE, lends itself particularly well to detailed determination of vinyl orientations.[9-11] The fractional change in intensity of spin i upon saturating spin j is given by[8] $\eta_{j\rightarrow i} = \sigma_{ij}/\rho_i$, where the cross-relaxation rate $\sigma_{ij} \propto r_{ij}^{-6}\tau_c$ (r is the distance between spins i and j and τ_c is the protein tumbling time) and ρ_i is the relaxation rate for spin i. For a 30° dihedral angle with the heme plane, r(CH$_3$-H$_\alpha$) ~ 4.3 Å and r(CH$_3$-H$_{\beta\text{-}t}$) ~ 2.1 Å for the cis and ~2.8 Å and ~5.2 Å for the *trans* orientation, respectively. The shorter distance in each case is consistent with the detection of a NOE in a protein.[8-11] In a strictly out-of-plane (perpendicular)

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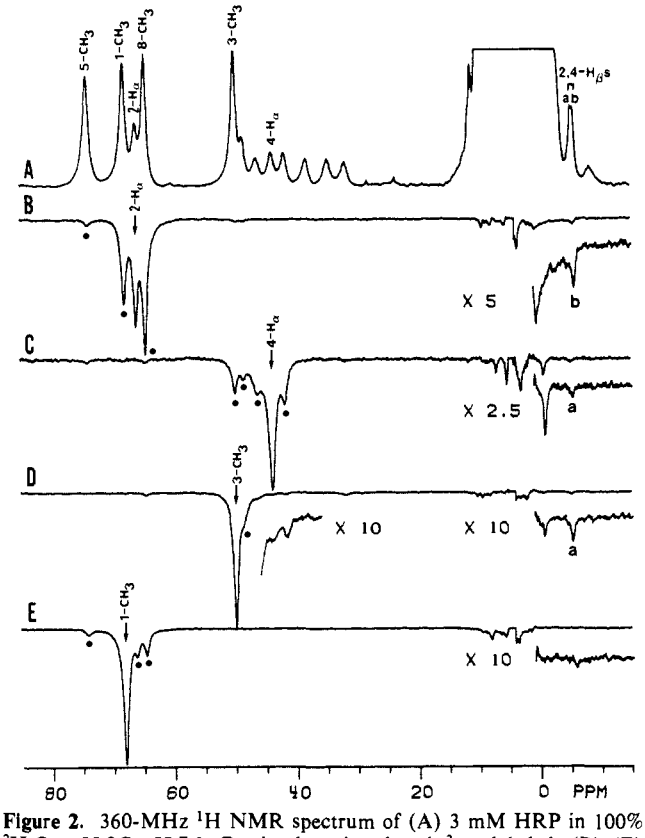

Figure 2. 360-MHz ^1H NMR spectrum of (A) 3 mM HRP in 100% ^2H_2O at 55 °C, pH 7.0. Previously assigned peaks[3] are labeled. (B)–(E) are the NOE difference spectra obtained as described in Figure 1. In each of the difference spectra, an arrow indicates the peak being saturated. (B) Saturate 2-H_α; note NOE to peak b. (C) Saturate 4-H_α; note NOE to a as well as to peak at −0.9 ppm. (D) Saturate 3-CH_3; note NOE to a. (E) Saturate 1-CH_3; note an absence of NOE to b. Difference peaks due to off-resonance saturation (power spillage) are marked as filled circles.

orientation, both distances are too large to yield significant NOEs. While such methodology has been applied to a small diamagnetic hemoprotein,[9-11] extension to larger paramagnetic proteins faces several potential obstacles, including inability to saturate effectively or selectively due to large line widths, extensive spin-diffusion in such large systems which obscures any primary NOEs,[12] and the degradation of NOEs by large paramagnetic contributions to ρ_i.[8,13] We demonstrate here that substantial primary NOEs are observable in both the highly paramagnetic ($S = {}^5/_2$) HRP as well as the unstable HRP-I which lend themselves to both peak assignment and structure determination.

The resolved portions of the ^1H NMR spectra[14] of HRP-I[15] and resting state HRP are illustrated in A of Figures 1 and 2, respectively. Individual heme methyl and vinyl H_αs have been identified by isotope labeling[2,3] in each case: resolved vinyl H_βs have been collectively identified[2,3] (peaks a–d and a,b in Figures 1 and 2, respectively) but not assigned to either individual group or proton. In HRP-I, saturation of b yields a large NOE to a but not to c or d as well as a small NOEs to 4-H_α (and 3-CH_3) but not to 2-H_α (trace 1B); the effect to 4-H_α is larger when saturating b than a. Similarly, saturating c (and, in part, d, due to overlap) leads to a NOE to 2-H_α but not 4-H_α (Figure 1, trace C); the effect to 2-H_α is larger when centering the decoupler on c than d (not shown). The fixed geometry of a vinyl group demands that a, b, c, and d arise from 4-$H_{\beta\text{-t}}$, 4-$H_{\beta\text{-c}}$, 2-$H_{\beta\text{-c}}$, and 2-$H_{\beta\text{-t}}$, respectively. Saturation of 1-CH_3 yields a 2% NOE to 2-H_α but none to either 2-$H_{\beta\text{-c}}$ or 2-$H_{\beta\text{-t}}$ (Figure 1, trace D); this dictates that the 2-vinyl group is close to in-plane and oriented *trans* (Figure 1F). In contrast, saturation of 3-CH_3 yields a sizable NOE to 4-$H_{\beta\text{-t}}$ but none to 4-H_α (Figure 1, trace E); this is consistent only with a near in-plane *cis* orientation (Figure 1F). In resting state HRP, saturation of 2-H_α (trace 2B) and 4-H_α (trace 2C) leads to NOEs to vinyl H_β peaks b and a, respectively. These connectivities, together with the different sizes of the NOEs, suggests a = 4-$H_{\beta\text{-t}}$ and b = 2-$H_{\beta\text{-c}}$. Saturating 3-CH_3 (Figure 2, trace D) leads to a large NOE to 4-$H_{\beta\text{-t}}$ and none to 4-H_α, confirming an in-plane *cis* orientation for the 4-vinyl group. Saturating 1-CH_3 fails to yield a detectable NOE to 2-$H_{\beta\text{-c}}$ (Figure 2, trace E), but a NOE to 2-H_α cannot be observed because of spectral overlap of the two peaks. Hence, the data are consistent with, but not proof for, a 2-vinyl *trans* orientation.

The absence of spin diffusion[12] in these large ($M_r \sim 42$ KD) paramagnetic protein complexes is witnessed by both the selectivity of the NOEs and by the essentially zero difference spectrum in the extremely intense diamagnetic region 0–10 ppm (Figure 2). Since spin diffusion is extensive in the less paramagnetic HRPCN[16] and diamagnetic Hb complexes,[17] it appears that paramagnetic relaxation is effective in short circuiting spin diffusion to the protein matrix[12] but not efficient enough to obscure any of the highly selective primary NOEs among heme substituents. Even with the 400-Hz line width, saturation is effective and power spillage effects are readily differentiated from NOEs by proper controls. The short T_1s (~8 ms) for the heme resonances allow very rapid data acquisition so as to make possible the detection of small NOEs even in relatively unstable reactive intermediates such as HRP-I. Since all methyl and vinyl H_α peaks are resolved in the ferric high-spin form of hemoproteins,[18] the present method has direct applicability toward determining vinyl orientations in any hemoprotein which yields a resolved spectrum. A correlation between the NMR-determined vinyl orientations and the vinyl RR bands[6,7] should allow a more useful and critical calibration of the latter method. Interpretation of RR frequencies in terms of vinyl orientation has already been questioned.[11] In fact, the present NOE methodology can serve as an important new tool for resonance assignments and structure determination in diverse classes of large non-heme proteins containing strongly paramagnetic ions.

Our determination of *trans* and *cis* orientations for the 2- and 4-vinyl groups in the functional resting state and compound I of HRP is in contrast to the RR proposals of *cis,cis* orientations in reduced HRP. The possibility that a vinyl group reorients during reduction or ligation, as well as more quantitative interpretation of the observed NOEs in terms of structure, is under current investigation.

Acknowledgment. This research was supported by a grant from the National Institutes of Health, GM-26226.

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(14) ^1H NMR spectra were recorded on a Nicolet 360-MHz spectrometer. Typical spectra consisted of (2–3) × 10^4 transients using a 6-μs 90° pulse and 8K data points over a 40-KHz band width with a pulse repetition time of 100 ms.
(15) HRP-I is the reaction intermediate in the enzymatic degradation of H_2O_2 by HRP.[2] HRP-I is generated by the addition of 2–3 equiv of H_2O_2 to resting state HRP. This green species is stable for 1–2 h at 15 °C.
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Book Reviews

Organotin Compounds in Modern Technology. Journal of Organometallic Chemistry Library. Volume 16. By Colin J. Evans and Stephen Karpel (International Tin Research Institute). Elsevier Science Publishers: Amsterdam and New York. 1985. X + 279 pp. $72.25. ISBN 0-444-42422-9.

Over the last three decades, organotin compounds have found increasing and widespread use in various technologies. Together with an increased number of research publications, many excellent books, monographs, and review articles have summarized chemical, physical, and structural aspects of this growing group of compounds, with only limited and sometimes cursory attention being given to applied aspects.

This obvious gap is now rather successfully bridged by a book, almost entirely devoted to technical applications of organotin compounds. Both important and well-established technical applications such as the use of organotin compounds as PVC stabilizers, as wood preservatives, as ablative marine antifouling agents, as agricultural chemicals, and as catalysts in polyurethane foam and silicone manufacture, as well as more experimental and recent applications in medicine and as protective coating agents, are extensively covered in well-structured and well-written chapters.

A brief survey of each technology is generally followed by detailed information on the use of organotins and their availability, supplemented by a reasonably critical evaluation of their effectiveness, with safety precautions and a brief summary on environmental effects included where applicable. All chapters are extremely well-referenced and good use is made of photographs and figures to illustrate specific aspects.

A separate chapter is devoted to the use of mono-oganotins. Finally, an attempt is made to summarize current views on the environmental effects of the organotins. In spite of a comparatively low toxicity level of most organotin compounds and their final inorganic degradation products, the large amounts of organotin compounds produced worldwide, currently estimated to be well in excess of 30 000 tons annually, are reason for concern, because most of them will find their way eventually into the aquatic environment. This aspect bears watching and may require more detailed attention than is given here.

As the title implies, this is principally aimed at technologists and applied chemists, but the academic researcher will find it a valuable sourcebook and extremely interesting reading.

F. Aubke, *University of British Columbia*

Methods in Enzymology. Volume 113. Glutamate, Glutamine, Glutathione, and Related Compounds. Edited by A. Meister (Cornell University Medical College). Academic Press, Inc.: Orlando, FL. 1985. xxxiii + 723 pp. $75.00. ISBN 0-12-182013-0.

The increasing awareness of the importance of this group of related biological compounds to metabolism, particularly with regard to xenobiotics, makes this volume a timely addition to a valuable series. The first section, composed of 26 chapters, covers glutamate metabolism. In addition to detailed descriptions of the methods developed for the purification and characterization of the enzymes involved in glutamate metabolism, chapters discussing γ-carboxyglutamic acid and the synthesis of folylpolyglutamates are also included in this section.

The second section of 20 chapters is devoted to glutamine metabolism. It includes topics ranging from the biosynthesis of glutamine to the utilization of glutamine-derived ammonia in a number of the amination reactions intrinsic to many biochemical pathways.

The subject of glutathione constitutes the third section of 28 chapters. This is the most far-ranging section in terms of the topics covered, reflecting the importance of glutathione to metabolism. Several chapters are devoted to the glutathione transferases which are involved in both drug and xenobiotic metabolism. Also included in this section are discussions of the enzymes which maintain the in vivo thiol–disulfide balance; methods for the detection and quantitation of cellular glutathione and glutathione disulfide; and methods for the preparation of γ-glutamyl amino acids. Of particular interest is the chapter outlining glutathione metabolism and the information obtained from manipulation of cellular glutathione levels. The final two sections of this volume, consisting of 7 and 2 chapters, respectively, deal with aspartate and asparagine, and with α-amino adipate.

This volume consistutes an excellent reference source of the current information available about this area of metabolism and should be a valuable resource to workers in this and related fields.

Carol A. Caperelli, *Fox Chase Cancer Center*

Structure and Bonding. Volume 62. Clusters. With contributions by F. A. Cotton (Texas A&M University), G. Gliemann (Universität Regensburg, F.R.G.), G. Schmid (Universität Essen, F.R.G.), R. A. Walton (Purdue University), and H. Yersin (Universität Regensburg). Springer-Verlag: Berlin, Heidelberg, New York, and Tokyo. 1985. 161 pp. $35.50. ISBN 0-387-15731-X.

This volume provides three selected review articles in transition-metal cluster chemistry. The title is somewhat misleading because a comprehensive coverage of cluster chemistry and physics is not intended and is not provided. For example, there is little mention of exciting new developments in bare metal cluster systems in molecular beam environments or of new theoretical procedures being developed to understand metal–metal bonding. Instead, the perspective provided is that of the inorganic chemist, emphasizing ligated transition-metal cluster complexes produced by synthetic chemical methods. Within this framework, the articles presented provide a nice sampling of recent developments, covered on a detailed level.

The first chapter, by F. A. Cotton and R. A. Walton, is Metal–Metal Multiple Bonds in Dinuclear Clusters. Material on ditechnetium, dirhenium, ditungsten, diruthenium, and diosmium cluster systems is presented, including developments in these areas since the publication (in 1982) of the text Multiple Bonds Between Metal Atoms, by these same authors. Highlights in other related areas of cluster chemistry are also included, such as theoretical studies of multiply bonded M_2 molecules and experimental techniques used to probe bond lengths and bond orders in M_2 systems.

Chapter 2, by G. Schmid, is entitled Developments in Metal Cluster Chemistry—The Way to Large Clusters. This article is perhaps the most useful part of the book for the nonspecialist. In introductory material, the simple building blocks of polyhedral systems are discussed (up to 13 atom structures). Other sections deal with synthetic methods to build larger clusters containing these units and with considerations of ligand selection to stabilize large clusters ($N > 13$). The discussions on skeletal arrangements of metal atoms and electron counting rules to predict and/or rationalize observed cluster stability will be relevant to a broad audience. The same will also be true for the section on newly synthesized large clusters up into the 50–60 metal atom size range.

The third review article in this volume, by G. Glieman and H. Yersin, is Spectroscopic Properties of the Quasi One-Dimensional Tetracyanoplatinate(II) Compounds. This is the most specialized and detailed review of the three and will therefore appeal to a more limited audience. Polarized luminescence spectroscopy and its dependence on temperature and magnetic fields are used to study the electronic properties of a variety of $M_X[Pt(CN_4)]$ complexes.

In a final section, this volume also contains the Author Index for Volumes 1–62 of the "Structure and Bonding" series.

Michael A. Duncan, *University of Georgia*

Drug Discovery: The Evolution of Modern Medicine. By Walter Sneader (University of Strathclyde). John Wiley & Sons, Inc.: New York. 1985. 435 pp. $21.95. ISBN 0-471-90471-6.

Through this book, the author tells a fascinating story on how man has progressed toward the achievement of an ancient dream—the conquest of disease through the use of effective drugs. He attempts to convey some of the drama and excitement experienced by the scientists and doctors who were involved. This historical account of the development of successful drugs is organized by therapeutic areas into 16 chapters. These include antibiotics, central nervous system depressants, cancer chemotherapy, vitamins, and cardiovascular drugs. The book is interspersed with chemical structures of the hundreds of compounds discussed, making it highly interesting to the chemist involved with drug discovery. The descriptive passages will interest those with very little chemical knowledge.

Each chapter has an extensive bibliography for those who wish to pursue the subject matter further. The index includes people, places, companies, and chemical names. It must have been a formidable task acquiring the basic facts needed for an effective book of this type.

Given the reasonable price, readability, and the informational content, this reviewer recommends this book to any person involved with chemistry or a health-related field. In this age of toxic waste and other ills of the chemical industry, it is refreshing to read about the good that has been achieved through chemistry.

James R. Zeller, *Warner Lambert/Parke Davis*