Origins of Biological Magnetic Resonance

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Nineteen ninety-six marks the 50th anniversary of publications describing the first detection of nuclear magnetic resonance (NMR)[1] signals [by Purcell, Torrey, and Pound at Harvard (1) and by Bloch, Hansen, and Packard at Stanford (2)]. The Nobel Prize for Physics was awarded to Bloch and Purcell in 1952 "for the development of new methods for nuclear precision measurements and the discoveries in connection therewith." The remarkable field that these discoveries spawned has moved hand in hand with the technological advances of the second half of the 20th century. In 1991, "contributions to the development of the methodology of high resolution nuclear magnetic resonance (NMR) spectroscopy" were cited in the award of the Nobel Prize for Chemistry to Richard Ernst. In his Nobel lecture, Ernst concluded, "I am not aware of any other field of science outside of magnetic resonance that offers so much freedom and opportunities for a creative mind to invent and explore new experimental schemes that can be fruitfully applied in a variety of disciplines" (3). Ernst's lecture also summarizes how this technique of the latter half of the 20th century grew out of developments in physics in the first half of the century.

Revolutions in magnetic resonance have continued during the 50 years since the first publications, and these revolutions have maintained a steady influx of new practitioners. I was an undergraduate at Stanford in the late 1950s, and it would have been difficult to avoid getting caught up in the excitement of the new discoveries on the campus and at nearby Varian. At that time, Felix Bloch turned out each fall to participate in the Physics Department picnic to welcome new physics students. The colorful chemistry professor, Richard A. Ogg, introduced me to NMR, and I remember his declaration: "Do you students realize what this is going to mean to the field of chemistry?" He had just delivered a lecture describing the first observation of resolved chemical shifts in ethanol (4) and his own measurements of proton transfer rates in liquid ammonia solutions (5). Scrupulously dry liquid ammonia gives a proton NMR signal of three lines because, although the three protons of ammonia are equivalent, they reside on nitrogens with one of three different nuclear moments. However, traces of water, in liquid ammonia, lead to reaction [1]; by which

$$NH_{3\ (liquid)} + H_2O \rightleftarrows NH_4^+ + OH^- \qquad [1]$$

protons lose track of the nitrogen nucleus with which they interact and thus give a spectrum of just one line (one of Ogg's results is illustrated in **Fig. 1**). A little knowledge of equilibrium constants and frequency units allowed us to calculate the rates of proton transfer between water and ammonia or amines. At the time of Ogg's work on proton and nitrogen NMR of amines, there was wide interest in rapid chemical reactions and how to measure them. His report (5) at the Faraday Society discussion on rapid reactions attracted much attention to NMR as a technique for investigating dynamics. Ogg's vision of the impact of NMR on chemistry was soon realized. The Varian bulletins of NMR spectra of organic compounds began to appear (6), and by the early 1960s, structure proofs of natural products as complex as taxinine (7) and tetrodotoxin (8) relied heavily on NMR data.

Resolution of the signals from hundreds of nuclei, sensitivity to structure, and responses to dynamics are the features that today make NMR a premier technique in structural biology (9, 10). The necessary first steps in biological NMR experiments followed along the lines of chemical ones. Oleg Jardetzky and colleagues made proton resonance assignments for monomers and dimers of biological polymers on the basis of chemical shifts and spin-spin couplings for amino acids (11, 12) and nucleotides (13). Phosphorus resonance assignments of ADP and ATP were made by Cohn and Hughes (14). Early in the process of studying biopolymers, it became apparent that information was also available in NMR spectra about secondary and tertiary structure. Kowalsky and Cohn (15) stated the case succinctly in their 1964 review. "Although its [NMR] application to biochemistry is still in its infancy, some very striking effects have been uncovered with molecules and systems of biological origin. Perhaps this is not too surprising since these molecules are optimally designed to carry out a given function, and consequently their electronic configuration about the nuclei and the configuration of the atoms within the molecule might be expected to exhibit characteristics at the extreme end of the range." The special manifestations of protein tertiary structure in NMR spectra were highlighted by McDonald and Phillips (16) in a 1967 paper describing unique chemical shifts of resonances in ribonuclease, lysozyme and cytochrome *c*. The observation of differential amide exchange rates, noted in early experiments with ribonuclease A (refs 16–18, and discussed in ref 15), was the ancestor of today's studies of protein folding. Ring current shifts were discovered in solutions of purines, thus providing evidence not only of base stacking interactions in solution, but also that the dispersion of chemical shifts in nucleic acids is enhanced by long-range structure (19).

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[1]Abbreviations: EMR, electron magnetic resonance; NMR, nuclear magnetic resonance.

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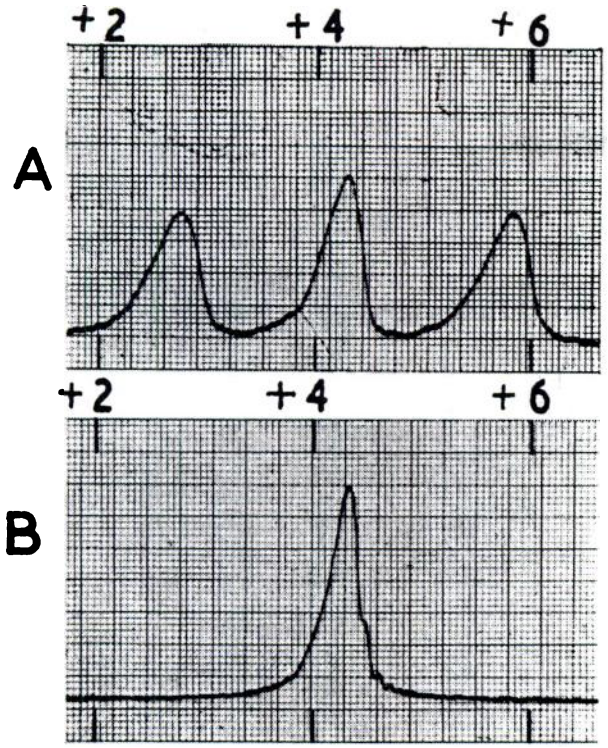

Figure 1. One of the first examples of the proton NMR spectrum of liquid ammonia is shown. In panel *A*, the liquid had been carefully dried; in panel *B*, it contained a trace of water. The presence of water resulted in the rapid equilibrium depicted in equation [1]. The spectra were recorded at 30 MHz and the numbers on the X-axis are displacements from the water resonance, in parts per million (from ref 5).

A new age in NMR studies of macromolecular structure was inaugurated with the reports on NMR studies of proteins obtained from algae grown in deuterated water (^2H_2O), supplemented with a selected, protonated amino acid (20, 21). Examining a protein one amino acid at a time was one approach to solving the resolution problem in NMR of macromolecules. Paramagnetic shifts of resonances from selected nuclei in metalloproteins afforded another possibility (22). But the goal of resolving the proton resonances of an isotopically unsubstituted protein could only be achieved with the Fourier transform revolution (23). The technological advances leading to NMR as it is currently practiced are nicely summarized in a historical review (24). Developing these advances into methods of macromolecular structure determination has taken single-minded dedication in a number of laboratories. Kurt Wüthrich began with his studies of cytochrome *c* (22) in the Shulman biophysics group at Bell Labs. John Markley began his studies of nuclease at Harvard and at Merck Sharp & Dohme with Oleg Jardetzky (21). **Figure 2** compares NMR spectra from Markley's studies of staphylococcal nuclease in 1968 (21) and today (A. P. Hinck, J. F. Wang, M. Kainosho, and J. L. Markley, unpublished results). The recent scientific biography (10) of Kurt Wüthrich provides an interesting historical account of his own work and that of others during the period of development of NMR methods for macromolecular structure determination. The protein inhibitor of α-amylase, Tendamistat, provided the first comparison of a protein structure determined independently by NMR (Wüthrich lab) and X-ray diffraction (Huber lab), and the close agreement of the structures established the credibility of NMR in protein structure determination (25).

The magnetic moment of the free electron is 658 times larger than that of the proton; the relaxation times differ by orders of magnitude, that of the unpaired electron being shorter. Consequently, it is easier to detect the resonant absorption of energy by electrons than by protons or other nuclei. An electron magnetic resonance experiment frequently precedes an NMR one in a developing branch of magnetic resonance. Credit for first observation of magnetic resonance actually goes to the Russian scientist E. K. Zavoisky (26) for detecting electron magnetic resonance (EMR, or in earlier literature, EPR, for electron paramagnetic resonance) in inorganic compounds in 1944 (published in 1945). Fifty years later, this achievement was celebrated in Kazan at the 27th Congress AMPERE held August 21–28, 1994 (27). Other examples of the electron magnetic resonance experiment preceding the nuclear counterpart include the definition of rates of motion of lipids in membranes by experiments with paramagnetic spin labels (28), followed by more detailed definition from NMR studies, and currently, developments using atomic force microscopy in a new approach to magnetic resonance imaging of very small samples (29, 30).

EMR of unpaired electrons occurring naturally in biological systems was under active study by the early 1950s. In a well-formed methemoglobin crystal, the orientation of the heme normal can be determined by EMR to an accuracy of about 1.5° by examining spectra of frozen crystals as a function of orientation. Thus, it was possible to determine the relative orientation of two pairs of heme groups in the methemoglobin tetramer (31, 32). EMR also revealed, in 1956, the presence of free radicals in photoreaction centers (33). Barry Commoner and colleagues began their paper on light-induced paramagnetism in chloroplasts: "oxidation-reduction processes, which play a pervasive role in the chemistry of living cells, necessarily involve the transfer of electrons. It has been proposed by Michaelis and others that biochemical electron transfers occur singly rather than in pairs" (33). Searching for evidence of single electron transfers, they found it when they observed an EMR signal from chloroplasts in the presence of light and measured decay of the signal in the dark. Theories developed in early studies of organic free radicals (34, 35) have been critical to understanding the origins of EMR signals in photosynthetic systems. Some highlights of characterizing the chemical and physical composition of bacterial photoreaction centers have included analysis of EMR signals of the "special pair" of chlorophylls in the primary donor, a manganese cluster, and quinone and tyrosine radicals. George Feher's (36) work on photosynthesis has continued from pioneering studies that identified the primary

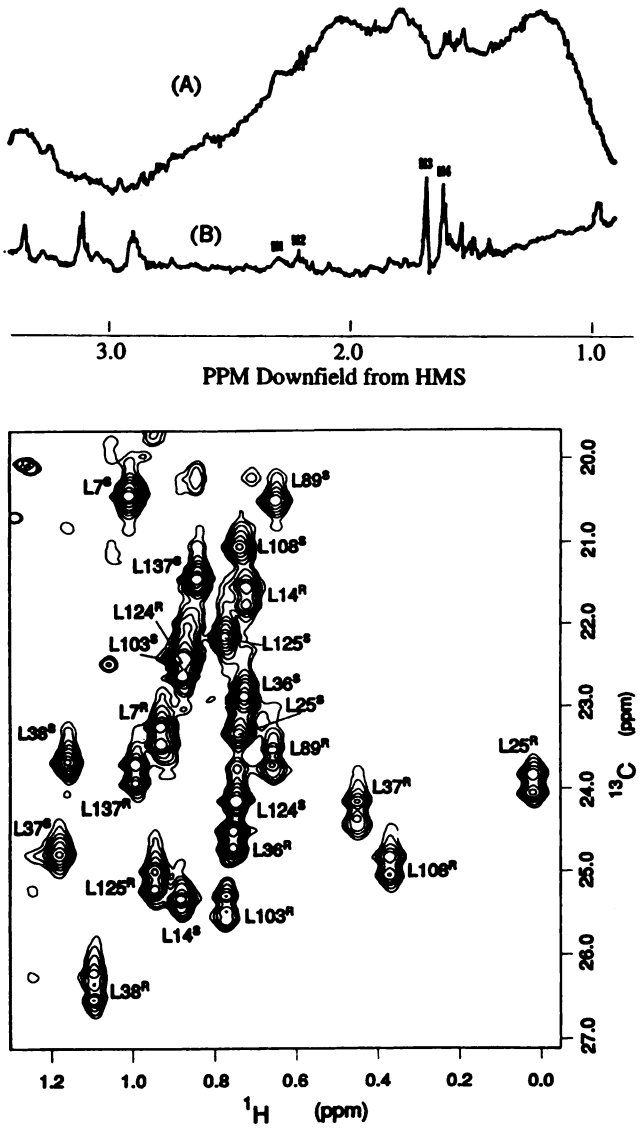

Figure 2. Examples are given of NMR spectra of staphylococcal nuclease from 1968 (upper, panels *A*, *B*) and from 1996 (lower) (from ref 21; Hinck et al., unpublished results, respectively). The 1968 spectra were acquired at the then state-of-the-art ^1H frequency of 220 MHz. *A)* Staphylococcal nuclease fully protonated except for sites that exchanged with the ^2H_2O solvent; *B)* an analog of the same protein prepared by growing the Foggi strain of *Staphylococcus aureus* on a mixture of ^1H and ^2H-labeled amino acids, with the only ^1H amino acids being methionine and tryptophan. Signals (M1-M4) from the methyl groups of the four methionines are resolved. The chemical shift scale is in ppm down field from external hexamethyldisiloxane (HMS) (redrawn from ref 20). The 1996 spectrum (lower) is of the same protein now produced recombinantly in *Escherichia coli*. In this case, the labeled amino acid incorporated was leucine with a dual labeling pattern: roughly half [$^{13}C^{\gamma}$-$^{13}C^{\delta}1$]leucine and half [$^{13}C^{\delta}2$]leucine. The net result is that in the methyl region of the 2-dimensional $^1H^{13}C$ NMR spectrum shown, all signals from *pro-R* methyls ($C^{\delta}1$) are doublets from carbon-carbon coupling, whereas all signals from *pro-S* methyls ($C^{\delta}2$) are singlets. The peaks are shown as contour plots of signal intensity. Here the chemical shift reference is internal 2,2-dimethylsilapentane-5-sulfonic acid (DSS). Note that the signals from the methyl groups of the leucines overlap one another; signals from the other amino acids are in the 1-dimensional proton spectrum of the protein at natural abundance (see the 0.0 to 1.2 ppm region of spectrum A, upper figure). The modern (500 MHz) spectrum illustrates how the technique of isotopic labeling, which is now highly practical because of advances in biotechnology, reinforces the technique of multidimensional NMR spectroscopy; together they yield higher resolution plus information for assignments. Complete sequence specific and stereospecific NMR assignments are now routine for small proteins.

photochemical reactants to the present, and Okamura and Feher (37) recently reviewed proton transfer in reaction centers. Tyrosine radicals also play a role in biological oxidation-reduction reactions and were first characterized in the enzyme, ribonucleotide reductase (38). High sensitivity also renders EMR useful as a probe technique. The use of extrinsic free radical probes, spin labels (39–41), in the laboratory of Harden McConnell at Stanford followed soon after the first report of synthesis of stable, aliphatic nitroxides by groups in Russia headed by O. L. Lebedev and L. B. Volardsky (42, 43).

From the beginnings of magnetic resonance touched on here, many modern innovations have arisen. NMR of solids and magnetic resonance imaging (MRI) deserve their own historical accounts. The former has required "taming" nuclear dipole-dipole interactions (44) over more than 25 years, whereas the latter rocketed from concept (45) to applications with living organisms almost immediately (46). A number of the new ideas in magnetic resonance have emerged from discussions at international meetings, for instance, the idea of 2-dimensional NMR was put forth by Jeener (47) at a summer school. The International Conference on Magnetic Resonance in Biological Systems brought together the many investigators who contributed to new developments, beginning with the first in this series, held in Boston in 1964, organized by Mildred Cohn, Oleg Jardetzky, and Robert G. Shulman. The proceedings of the second conference in this series, organized in Stockholm in 1966 by Anders Ehrenberg, Bo Malmström and Torr Vänngård, was published (48) and provides a historical account of the directions that many practitioners of the art were following at that time. The XVIIth meeting in this series was held this year at Keystone Colorado, August 18–23, and was organized by Andrew Byrd, Ad Bax, and Ted Becker.

The helpful suggestions of John L. Markley, who read the manuscript, and with his colleagues A. P. Hinck, J. F. Wang, and M. Kainosho provided the unpublished, modern spectrum of staphyloccocal nuclease shown in the lower part of Fig. 2, are gratefully acknowledged. The author learned magnetic resonance with R. A. Ogg, H. S. Mosher, K. Nakanishi, and H. M. McConnell. On this occasion, it is a pleasure to dedicate this article to Koji Nakanishi, who recently celebrated his 70th birthday.

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