BIOLOGICAL APPLICATIONS OF RAMAN SPECTROSCOPY

VOLUME 1: Raman Spectra and the Conformations of Biological Macromolecules

THOMAS G. SPIRO, EDITOR

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VOLUME 1

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Preface

Some 60 years have passed since the discovery by C. V. Raman (1) of the effect that bears his name. In the decade following this discovery, Raman spectroscopy provided data on the vibrational frequencies of many molecules (2). Only during the 1940s did the introduction of practical infrared (IR) spectrometers lead to the displacement of the more laborious Raman method for routine recording of vibrational spectra. In the prelaser days, heroic measures had to be taken to record the very weak spectra emanating from the Raman effect; this generally involved the placement of a large optically clear sample inside a high-intensity discharge lamp (3). The concentration of light power provided by the laser has revolutionized Raman technology (4) and vastly increased its applicability.

Biological molecules have historically been problematic as an arena for vibrational spectroscopy, despite the promise of this method as a probe of molecular structure. Infrared spectroscopy is constrained by the fact that water, the ubiquitous solvent of biology, is a strong absorber of IR radiation. It is a weak Raman scatterer, and, as early as 1938, J. T. Edsall expressed the cautious hope that Raman spectroscopy could be applied to the study of proteins (5). Twenty years elapsed before this hope was rewarded with a "quite faint" photographic Raman spectrum of lysozyme (6). The first laser Raman spectra of proteins were published in 1968 (7). Since then the growth of the literature on biological applications of Raman spectroscopy has been explosive.

This set of three volumes provides a comprehensive view of a still new and rapidly evolving field through summaries of representative areas by authorities in biological Raman applications. A major research objective

has been to extract information from Raman spectra about the conformations of biological macromolecules: proteins, nucleic acids, and lipids. This is the theme of Volume 1, which considers these macromolecules in isolation and also in organized biological assemblies: ocular lenses, viruses, and membranes. In addition, the collective vibrational modes of macromolecules are considered from a theoretical standpoint, and there is a review of the promising new area of vibrational optical activity.

The introduction of the laser actually had two revolutionary influences on Raman spectroscopy. One was the dramatic relaxation of constraints on the size and optical clarity of the sample and the attendant improvement in signal quality. The other was the ability to produce Raman scattering from absorbing samples, thanks to the minimization of the light path allowed by the directional laser beam. This permitted systematic studies of the resonance Raman effect, which has been an extremely active and productive area from both experimental and theoretical perspectives. The importance of the resonance Raman effect for biological applications is that it can provide dramatically increased sensitivity and selectivity. The theory of the effect is presented in Volume 2, which also explores applications to rhodopsin, to the purine and pyrimidine bases of nucleic acids, to the peptide backbone and chromophoric side chains of proteins, to flavins, and, finally, to the introduction of resonance Raman labels into biological systems. Heme proteins have provided a particularly rich opportunity for resonance Raman studies, and various aspects of work in this area are discussed in Volume 3, which also includes applications to chlorophylls and nonheme metalloproteins.

It is hoped that these volumes give a sense of the high promise that Raman spectroscopy holds for providing unique and important molecular information in biology. They are dedicated to Professor Richard C. Lord, who, perhaps more than any other single individual, has been responsible for the development of this realm of science. His pioneering studies on proteins and nucleic acids have been an inspiration to most current practitioners in the field, and the students that he trained have played an important role in pushing back its frontiers. He is held in the highest esteem by his many friends and admirers, who respond to his wisdom and to his wonderful human qualities. One of his former students, Professor George Thomas, has written the Dedication that follows this Preface.

THOMAS G. SPIRO

Princeton, New Jersey
January 1987

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Dedication to Richard Collins Lord

Richard Collins Lord was born on the tenth day of the tenth month of the tenth year of this century and recently celebrated his seventy-sixth birthday. These volumes are dedicated to Professor Lord in honor of his long and distinguished career as a molecular spectroscopist and teacher (1), and particularly in recognition of his outstanding contributions to the field of Raman spectroscopy of biological molecules. It is a privilege and a pleasure to contribute these prefatory remarks acknowledging the pioneering work of Richard Lord and his colleagues in the development of Raman and infrared (IR) spectroscopy for biological research.

Lord's contributions to the discipline on which these monographs focus are remarkable for their scope and number; they are referenced repeatedly throughout the various chapters. His interest in the application of vibrational spectroscopy to biological systems began in the late 1950s, with his analysis of the hydration of calf thymus DNA by IR absorption spectrophotometry. These studies led to the publication of landmark papers by Falk et al. (2) on the effect of the hydration sphere on DNA secondary structure. The feasibility of Raman spectroscopy as a probe of DNA and RNA structure was demonstrated shortly thereafter in the series of papers by Lord and Thomas (3). Still widely cited, these papers of the 1960s reflect the originality that has always characterized Lord's work and the skill with which he has inspired his students and postdoctoral coworkers to gather and interpret reliable data from biological specimens of the highest available purity. As reliant as the present-day Raman spectroscopist is on the power and versatility of the laser, it is easy to overlook the fact that the initial studies on synthetic DNA and RNA analogs were carried out with the cumbersome mercury Toronto arc designed for the original Cary-81 recording spectrophotometer.

The first interpretable laser Raman spectrum of a native protein was obtained from lysozome by Lord and Yu (4), who also analyzed the Raman spectra of native ribonuclease and chymotrypsin. These investigations led to the realization that a number of important structural correlations could be derived for both side and main chains of proteins. Especially noteworthy are the original assignments of the amide I and amide III frequencies of alpha and beta conformations and their variation with protein denaturation (4,5) and the quantitative interpretation of the now well-known tyrosine doublet in terms of hydrogen bonding states of the phenoxyl group (6). The latter study represented a collaboration between Lord's group at the Massachusetts Institute of Technology and the University of Tokyo group of Lord's long-time friend and colleague, the late Professor Takehiko Shimanouchi.

Several important advances were made by Lord and his associates in the application of vibrational spectroscopy to DNA and RNA constituents in

the late 1960s. The elegant IR spectrophotometric studies of Kyogoku et al. (7) demonstrated the stereospecificity of hydrogen bonding between purines and pyrimidines in solutions of monomeric DNA and RNA nucleotide analogs. The spectroscopic data were also analyzed to reveal, for the first time, the thermodynamic parameters governing base-pair formation in the absence of base stacking interactions. These studies developed from a technique that was devised by Lord and Porro in 1960 (8) and applied to A–U base-pairing in 1965 by Hamlin et al. (9).

As sample quantities of biologically active nucleic acids became more generally available in the last decade, Lord turned a greater part of his attention to the exploitation of Raman spectroscopy for studies of both RNA and DNA. In a paper that exploited one of the unique virtues of Raman spectroscopy, Chen et al. (10) showed that the structure of yeast phenylalanyl transfer RNA in aqueous solution was the same as that in the crystal that had been solved by x-ray diffraction analysis. Later use of the same methodology demonstrated that the x-ray crystal structure of Z-DNA was similar to the aqueous solution structure (11).

In his tribute to Lord 10 years ago, Professor Foil A. Miller, who was Lord's first Ph.D. student, wrote:

> No list of his contributions would be complete without mention of one of the most important of all—the influence he has had through the training of people. This has been multifaceted: through formal teaching of undergraduate and graduate classes, by directing graduate studies, by having guest workers in his laboratory, and through his summer infrared courses. He has been eminently successful. His relations with his graduate students were excellent. It was his custom to eat lunch with them regularly at a large round table in the student refectory. This was a splendid educational technique, transferring a great deal of information informally and developing the *esprit de corps* of his group. There was a fine spirit of camaraderie and a genuine pleasure in the work. Lord is an excellent teacher who strives to give his students understanding and insight in addition to hard facts.

I have yet to meet a student, colleague, or acquaintance of Lord's who would dispute the letter or spirit of Miller's tribute. Richard Lord is a great scientist and—more importantly—a great person. He has served all of us as he has served science. With respect and affection we dedicate this work.

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January 17, 1987

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Peptides and Proteins

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Contents

1 INTRODUCTION

The three-dimensional structure and the intramolecular–intermolecular interactions of a molecule determine the frequencies and forms of its normal modes of vibration. The frequency shifts observed in Raman scattering (viz., inelastic scattering of monochromatic, usually visible, light) correspond to such normal-mode frequencies; therefore, this spectroscopic technique has been widely used to obtain information on these molecular properties and how they are influenced by environmental factors.

In the case of small molecules, the structure and forces can often be derived directly from vibrational spectra [Raman and infrared (IR)] (1), since sufficient data are available. For large molecules, however, this is not feasible; therefore, the favored approach has depended on the development of correlations between observed bands and known chemical—and occasionally physical—structural features of similar classes of molecules (2). This was effective because, although all atoms in a molecule usually participate in a vibrational mode, for certain chemical groups of atoms (e.g., CH, NH, C=O) the molecular motion is highly concentrated in the group and is essentially unchanged from one molecule to another. The disadvantage of this group frequency approach is that it is necessary to have independent knowledge of the specific chemical or physical structure being studied, and thus unfamiliar spectral features are difficult to interpret in terms of structural and environmental variations.

Nevertheless, when the first laser Raman spectra of several crystalline proteins were reported (3), it became clear that Raman spectroscopy of peptides and proteins would become a powerful tool in elucidating structural questions involving such molecules. An important advantage of Raman scattering over IR absorption is the ease with which spectra of aqueous solutions can be obtained (because Raman scattering by water is weak). The utility of the group frequency approach in such studies was soon demonstrated in the seminal papers of Lord and Yu (4), who showed how the Raman spectra of lysozyme, ribonuclease, and α-chymotrypsin could be interpreted in terms of their constituent amino acids. In subsequent studies, amino acid and peptide spectra were catalogued (5), the general approach was extended to polypeptides (6–8), and applications to proteins were expanded significantly (7,9–13). In the latter area, methods have also been proposed for using Raman spectra to determine secondary structure composition in globular proteins (14,15).

Such studies have been concerned mainly with nonresonant Raman scattering, in which the frequency of the exciting light is far from an