Physical Methods for Inorganic Biochemistry

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Preface

This volume is intended for students and professionals in diverse areas of the biological and biochemical sciences. It is oriented to those who are unfamiliar with the use of physical methods in studies of the biological elements. We hope the reader will find the material a helpful reference for other volumes of this series as well as the general literature, and some may see ways to adopt these techniques in their own pursuits. Every effort has been made to avoid an abstruse presentation.

It should be clear that one individual cannot be expert in all the disciplines considered here (and the authors recognize that fact with sincere humility). As may be expected of an introductory reference, most of our attention was focused on the commonly used methods. To balance this, we have included a few examples of approaches which are promising but relatively undeveloped at this time. Also, an emphasis has been placed on element selectivity. It is impossible to envision the course of future events, and a volume which deals with instrumentation is especially prone to become outdated. Nevertheless, any valid approach to a scientific question should be applicable indefinitely.

Since there are four authors, the portions prepared by each should be identified. Chapter 6 (X-ray diffraction) was written by Wayne Hendrickson, Ph.D. Gordon James, Ph.D., prepared Chapters 11 (microprobe methods) and 12 (neutron activation analysis), while Shigemasa Osaki, Ph.D., contributed Chapter 10 (kinetic methods). John Wright, Ph.D., is responsible for the remaining material. We especially wish to recognize our wives for their support throughout this effort.

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critical reading of Chapter 6. Steven Sheriff made especially valuable contributions to Table 6-2. He is also grateful to Janet Smith for Figure 6-6, to Jane Richardson for Figure 6-7, and to Alwyn Jones for Figure 6-8. Wayne Hendrickson's portion of the manuscript was supported in part by a grant, GM29548, from the National Institutes of Health, and it was written while he was a member of the Laboratory for the Structure of Matter at the Naval Research Laboratory in Washington, D.C.

Contents

1 Introduction

In attempting to write a book treating the physical methods and their application to the special problems associated with the biological elements (Raymond, 1977), several questions were raised about what should be included. All of the known techniques are applicable to some degree, but if one conducts even a cursory inspection of the literature it will be apparent that there has been no lack of imagination in applying both old and new tools to the study of biomolecules and their interactions. A respectable literature accumulates rapidly in all areas, and the total effect is a deluge of information. Broad treatment of all these subjects goes well beyond the space and purpose of a single book.

Careful consideration of the types of evidence one may obtain from the various approaches will cause one to realize that all methods are not equal in their ability to yield unambiguous information about the behavior of a given type of atom or a group of atoms within a biomolecule; that is to say, some methods are more specific than others. (Some techniques, e.g., infrared spectroscopy, are virtually prohibited in the aqueous environment.) For example, light scattering experiments will provide estimates of the sizes and shapes of protein molecules. We might use these techniques to characterize a conformational change which occurs when a certain enzyme encounters and binds an activating metal ion. However, any observed changes will reflect geometrical alterations on the scale of the macromolecule, and may involve rapid equilibrations between two or more discrete conformational states.

This sort of evidence is by no means impertinent, but it does not provide much insight concerning the metal ion and its immediate environment. Contrast this situation with a ^{39}K-nuclear magnetic resonance experiment in which the potassium ion is observed to interact with a biomolecule. One is now receiving information from the ion itself in the form of chemical shifts and relaxation times (i.e., linewidth information),

and from such data a picture of the ion and its coordination environment may be formed.

Clearly, some methods are better suited than others for answering the questions of the bioinorganic chemist. We have thus chosen to emphasize techniques which discriminate between the different elements. We might ask, for example, why does the enzyme glutathione peroxidase contain selenium? What is so unique about a selenium atom that the evolutionary process has chosen it over all other atoms for this particular role? To answer our inquiry we will need to probe the selenium part of this enzyme in detail, e.g., to determine its valence and charge density, bonding environment and ligands, etc. It will be particularly informative to observe how these properties change when the enzyme interacts with a substrate. The enquiry is thus one into fine structure and dynamics, and it will require resolution on the scale of individual atoms. This is chiefly the domain of molecular spectroscopy and diffraction methods.

The design of the book has been to include in each chapter a discussion of the type of information one may obtain from the method in question. Limitations have been given special consideration. Much misinformation has been permitted to enter the literature out of inattention to the restrictions inherent in the method in use *and* the biological system under investigation. Each chapter contains an introduction to the method and its associated instrumentation, and the potential applications are illustrated by citing examples from the primary literature. The latter will hopefully convey to the reader the idea that physical instrumentation—however sophisticated or expensive—is fairly inert; as in all areas of science, there is no substitute for ingenuity and care in the design of an experiment.

A cardinal rule unifying these techniques is to avoid placing overreliance upon any one method. Evidence from two or more sources is often complementary, and this has been brought out in many places. Also, on the biological side of the question we should be aware of the highly coherent or synergetic quality of the interactions taking place within living entities. The nonequilibrium thermodynamic models of ordering phenomena (Haken, 1977) provide a quantitative basis for the more intuitive holistic views expressed by biologists for many years. When we reduce living cells to their component parts many significant characteristics will be lost. At the protein level there is always the possibility of artifactual behavior. In keeping with this concept, the illustrative examples range from "simple" one-protein cases through supramolecular aggregates to *in vivo* conditions. Fortunately, some of the techniques allow an examination of the living state. Less emphasis has been given to model systems even though these are indispensable in providing points of reference to more established chemical knowledge.

1.1 Physical Methods

In Figure 1-1 the major physical methods are grouped from left to right as their emphasis progresses from individual nuclei, through more complex structures, to the molecular entity. One will observe that the unifying principles of symmetry and group theory (Bunker, 1979; Cotton, 1963) bridge between these experimental tools and the chemical bonding concepts. It is at this level of interaction between data and theory that chemical insight is gained. All three areas (chemical theory, symmetry, and experimental methodology) are equally important, and the individual who hopes to use the physical methods must be aware of this fact.

Each physical method is, in the correct sense, a communication channel (Shannon and Weaver, 1949; Brillouin, 1964) between an observer and an atomic or molecular structure under investigation. In every case a source or *information carrier* (e.g., a light beam, electron beam, or radio frequency field) is *modulated* when it interacts with a sample of matter. Chemical information is thus coded upon the carrier.

Unfortunately, the information emerging from currently available in-

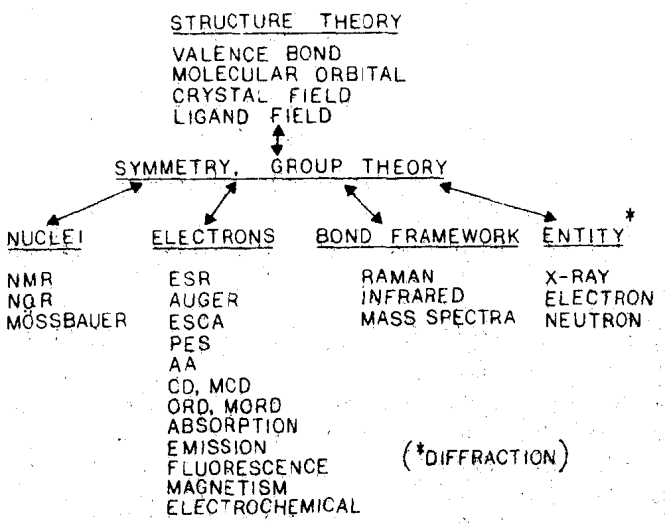

Figure 1-1. Relationships between the various physical methods and chemical theories. Abbreviations: ORD, optical rotatory dispersion; CD, circular dichroism; MORD, magnetic optical rotatory dispersion; MCD, magnetic circular dichroism; ESCA, electron spectroscopy for chemical applications; PES, photo-electron spectroscopy; AA, atomic absorption; ESR, electron spin resonance; NMR, nuclear magnetic resonance; NQR, nuclear quadrupole resonance.

strumentation is not of the direct variety and requires further *processing* in the perspective of chemical theory before one is able to visualize molecular features (e.g., geometry, electron densities, bond polarities, etc.). Also, some of the methods produce relatively more information in binary bits per data set than others, and it is possible to classify them (roughly) as channels of high, medium, or low content. On this scale the technique of X-ray diffraction would rate high while electrochemical methods would be relatively low.

The orientation of this volume introduces an additional criterion of comparison: element selectivity. Table 1-1 and the following discussion attempt to evaluate this important property.

1.1.1 Nuclei

The nuclear resonance methods fall into the medium to high range of channel capacities. Moreover, these methods are element selective, which is a simple consequence of the fact that it is the nucleus which determines an atom's identity. Mössbauer (Gonser, 1975) and nmr spectroscopy (Berliner and Reuben, 1978; James, 1975) have virtually absolute selectivity since natural linewidths and molecular effects upon line positions are quite small in relation to the spectral differences between the various isotopes. Nqr spectroscopy (Semin *et al.*, 1975) is more ambiguous since the chemical environment has a much larger effect upon line positions, although uncertainties may be removed by isotope replacement experiments.

1.1.2 Electrons

The electron-related methods are mostly medium-capacity information channels. They are also less selective than the nuclear resonances, a result of the many pairwise interactions experienced by molecular bonding and nonbonding electrons. However, under some circumstances one may be certain that the electronic property depends upon a particular type of atom. For example, if chemical assays have established that a protein contains a transition element it is reasonably safe to associate any observed paramagnetism with that element. If the protein yields an esr spectrum, one would also attribute it to the transition element; furthermore, the esr spectrum may contain information in the form of electron–nuclear coupling interactions which reveals the identity of the nu-

Table 1-1. A Comparison of Spectrometric Methods

Spectroscopic method	State	Region probed in the biomolecule
Nmr	*in vivo, in vitro*	Depends on the nucleus chosen. Abundant nuclei yield evidence from broad regions of the molecule, e.g., ^{1}H, ^{14}N, while a lone nucleus, e.g., a metal ion, may probe only a localized region.
Nqr	Crystal	Similar to nmr, but crystal-induced asymmetry may create more than one environment for the same nucleus, leading to a greater number of lines than equivalent nuclei.
Esr	*in vivo*, crystal, glassy	The region probed depends on the extent of delocalization of the unpaired electron(s), usually small (one atom) to moderate (several atoms).
Mössbauer	Crystal, glass	Small, usually the immediate environment of the ^{57}Fe atom.
Diffraction methods	Crystal	Whole molecule. These methods are difficult, but they are presently the only means for deriving an unequivocal space-filling structure.
ESCA	Crystal, amorphous	Whole molecule. Energy levels are grouped so that it may be possible to identify atom types. Works best when the atom of interest is present as a minor component of the macromolecule, e.g., a transition metal ion in a metalloprotein.

cleus or nuclei associated with the unpaired electron (Knowles *et al.*, 1976). Note again the desirability of observing nuclear-based phenomena. In other cases an unpaired spin may be more delocalized, as in the example of the free radical derivative of vitamin E (Kohl *et al.*, 1969) shown in Figure 1-2.

Selectivity is greatly increased when the electron involved in the spectral effect belongs to one nucleus in an isolated atom (e.g., atomic absorbance) or is one occupying an inner shell and therefore is influenced by a particular nucleus (e.g., X-ray fluorescence). Of the electron spec-