led to a narrower ^{67}Zn NMR with a half-band width of 12 Hz. By adding 1 m*M* bovine insulin to the 50 m*M* $ZnCl_2$ solution, at pH 2.95, the half-band width of ^{67}Zn NMR increased by three times. Addition of insulin more than 1 m*M* led to a broader ^{67}Zn NMR. The determined T_1/T_2 values of the 2 *M* and 50 m*M* $ZnCl_2$ solutions are almost unity. But, the T_1/T_2 ratio of ^{67}Zn NMR of the Zn^{2+}-insulin complex is not unity (1.36). From these findings, the correlation time, τ_c, and the quadrupole coupling constant are evaluated. The halfband widths of ^{67}Zn NMR are temperature dependent, when Zn^{2+} is bound to polymeric ligands. It is suggested from the temperature dependence that molecular weights of the ligands are closely correlated with the mechanism of the ^{67}Zn NMR of the Zn^{2+} complexes. The relaxation mechanism on the ^{67}Zn NMR will be discussed.

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B31

X-Ray Absorption Spectrometry as a Tool for the Study of Molecular Structure

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X-ray absorption spectroscopy is a relatively new tool for the investigation of electronic structure and the local environment of specific atoms in biological molecules. Until recently, these techniques were not practical for the low concentrations required in biological preparations. With the development of high flux X-ray beams produced by synchrotrons, much interest has been generated in development of its application to biological problems. It is a unique tool in that it probes only the local environment of specific atoms and is not limited by the physical state of the sample (*e.g.*, gases, liquids, solids, solutions, gels, etc.). Unlike X-ray diffraction, the entire structure of a crystal need not be solved to obtain information concerning the structure of the active site of a molecule. In these respects, X-ray absorption spectroscopy is ideally suited for biologically problems. It has grown enormously as a practical technique since the opening of the Stanford Synchrotron Radiation Laboratory in 1974.

In many biological systems the active sites include one or more transition metal atoms. In most cases, however, the metal atoms are very dilute and are responsible for only a small part of the total absorption. Fluorescence detection is usually the preferred measurement technique, and one is often forced to work with spectra of less than ideal signal-to-noise quality. Mathematical techniques have been developed to extract information from this type of experimental data. A further complication is that care must be taken to avoid X-ray damage of the biological samples.

EXAFS (extended X-ray absorption fine structure) experiments have been performed on a large number of macromolecules of interest to biochemistry. These experiments can roughly be divided into two groups. The first involves proteins where the ligands of the metal ion are already known from diffraction, and EXAFS is used to obtain more accurate bond length and geometry information. The second group of experiments deals with proteins where some or all of the ligands are unknown, and the goal is to determine the coordination number, the bond lengths, and ultimately the local structure. In both these groups model compounds play an important role in aiding the analysis and establishing confidence in the results.

Absorption features below the K X-ray absorption edge of 3*d* transition metal complexes can be assigned, in sufficiently ionic sites, to transitions from the 1*s* orbital to vacant 3*d*, 4*s*, and 4*p* orbitals. The spacing between these levels can be perturbed from those obtaining in the analogous free ion states since the vacant orbitals are hybridized with the filled orbitals of ligand atoms, leading to antibonding orbitals of elevated energy. This hybridization is, of course, quite different for the *d*, *s*, and *p* orbitals as they are of quite different sizes and symmetries. A study of a number of Cu(I) and Cu(II) complexes shows that the $1s \rightarrow 4s$ and $1s \rightarrow 4p$ ranges of observed energies of the features of the two charge states partly overlap, indicating that effects of covalency outweigh effects of valency itself.

In addition, there are multiple scattering modes in this region of the spectrum which, if properly interpreted, can give information on the local structure around the metal atom. Series of metal–ligand complexes in which the total charge state is changed while keeping the ligand atoms constant show how these atomic and scattering features depend on local structure.

Several examples of metalloproteins studied by the Bell Laboratories group will be described.
