Anisotropy and NMR of macromolecules

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A new approach for studying high molecular weight macromolecules and macromolecular complexes is coming to the fore. Such biological systems have traditionally been very difficult to structurally characterize, because of long global correlation times, light scattering, difficulty to crystallize, and more. For biological solid state NMR these are either advantages or at the least they are not serious problems. While this spectroscopy has been around for more than two decades, due to the complexity of the instrumentation and the spectroscopy only a modest number of functional questions have been effectively studied with this approach. Now these excuses for avoiding solid state NMR are fading and exciting data relating to functional properties of macromolecules are being generated by those using the technique.

Biological solid state NMR spectroscopy has been pioneered and promoted over the years by scientists such as: Myer Bloom (Nezil and Bloom, 1992), Robert Griffin (McDermott et al., 1991), Eric Oldfield (Oldfield, et al., 1991), Stanley Opella (Bechinger et al., 1992), and Jacob Schaefer (Marshall et al., 1990) to name a few. Many others, including Christopher Dobson (Taguchi et al., this issue) and Charles McDowell (Challoner et al., this issue) have made significant contributions to the development of this form of NMR for biological studies. 'Solid state' in solid state NMR refers to the observation of nuclear sites in anisotropic environments which may or may not mean a dry preparation. When the nuclear spin interactions are not averaged to their isotropic value, broad line spectra result, the original differentiating characteristic for this form of NMR. Techniques of 'magic angle sample spinning' and 'high power proton decoupling' are effectively used to recover the appearance of high resolution spectra, by 'averaging' the orientational dependence (with respect to the magnetic field) of the nuclear spin interaction tensors. Furthermore, by judiciously choosing the rate at which the sample is spun it is possible to obtain the magnitude of the chemical shielding tensor elements, and hence gain back the information which would have been lost due to rapid magic angle spinning. By this rather convoluted approach the sensitivity gained by 'focusing' the spectral intensity from a wide-line spectrum into an isotropic resonance is only partially lost through the generation of a set of spinning sidebands. Consequently you can have your cake and eat it too; you can have the sensitivity enhancement and have the characterization of the spin tensor. Two papers reported in this issue (Taguchi et al.; and Challoner et al.) utilize these techniques to observe ^{31}P spectra of pyridoxal phosphate in glycogen phosphorylase to assay the ionization state of the phosphate group.

These papers represent a rather elegant and simple use of solid state NMR to elucidate an answer for a long-standing functional question. Glycogen phosphorylase has been the subject of many structural studies, but the correspondence between crystal structures and solution observations continues to be the subject of considerable debate. In particular the ionization state of PLP in the T and R forms of glycogen phosphorylase b is unresolved. Earlier efforts to characterize the ionization state have utilized isotropic chemical shifts. These shifts represent the average of the chemical shift tensor elements and this tensor reflects the chemical shielding of the ^{31}P nucleus and hence the electronic charge distribution about this site. In the past ^{31}P chemical shifts have been correlated not only with the charge distribution but with various features of the covalent geometry of the phosphate. Certainly, alterations in the chemistry of the phosphate through changes in esterification or protonation will affect the tensor and its average value. However, the average is an indirect measure of the property of interest and a full tensor characterization requires solid state NMR and considerable background knowledge.

This background knowledge is the result of tensor characterizations of model compounds and without it the confidence level for the interpretation of the macromolecular data is poor. Here, these two studies contribute to the data base on model systems and it is clear that a very substantial difference exists in the tensors for monoanionic and dianionic phosphates. Furthermore, the tensor orientations from single crystal studies provide the necessary information for evaluating motional averaging schemes that could alter the tensor element magnitudes. For phosphate model compounds and for many other molecular systems this background data has been accumulating to the point where macromolecular studies can be interpreted.

While solid state NMR has the potential to provide detailed characterizations for the structure and dynamics of macromolecules and their complexes, it also shows a complexity that is often very difficult to interpret. The resolution of structural versus dynamical effects on an interaction tensor is very difficult to achieve, especially where the experimentalist's ability to manipulate the sample conditions is so limited, as it is for biological macromolecules. Here through an analysis of the ^{31}P chemical shift tensor it has been possible to ascertain that the T state is dianionic and that the R state is not

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purely monoanionic, but may represent a dynamic averaging of di- and monoanionic states. As we start to learn more about protein dynamics we may, in fact, find that such 'instability' is a typical characteristic of enzymes in their active form. We have recognized for many years that dynamics play a critical role in enzymatic activity and yet while we know a great deal about the protein fold of enzymes we know very little about molecular dynamics on a timescale relevant to chemical processes. Solid state NMR provides a unique opportunity to achieve such characterizations, because global motions do not dominate the spectral density functions for samples in anisotropic environments. The two papers reported in this issue of the Biophysical Journal represent a new wave of interest and application of solid state NMR methods for studying structure-dynamics-function relationships in macromolecules.

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