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A New Way to Assign ^{31}P Chemical Shifts

Phosphorus-31 nuclear magnetic resonance (NMR) spectrometry promises to be a valuable tool for studying the conformation of macromolecules such as DNA, RNA, and phospholipids. The reason, says Philip Bolton of Wesleyan University, is that the chemical shift of the ^{31}P signal may be dependent on torsional forces at the phosphorus-oxygen bond. The technique has not been used to any significant extent, however, because it has been very difficult to associate each signal with a specific phosphorus in the macromolecule. "There are about 35 macromolecules whose ^{31}P spectra have been published," Bolton says, "and virtually every possible assignment for each signal has been made."

In NMR spectroscopy with protons or carbon-13, this problem can usually be overcome because of the large catalog of known chemical shifts available. If this does not work, it is usually possible to replace one or more atoms with an isotope that does not have a magnetic dipole moment and therefore does not give an NMR signal. Replacement of a proton with deuterium, for example, removes the signal of the target atom from the spectrum, and thereby provides unequivocal identification. There is no comparable catalog of chemical shifts for ^{31}P compounds and the only available isotope, ^{32}P, has both a high radioactivity and a short half-life. Some assignments can be made if resolved and assigned ^{1}H NMR spectra are available, but this is a difficult and tedious process.

Bolton, John Gerlt of Yale University, and Ming-Daw Tsai of The Ohio State University have recently independently found a technique to assign chemical shifts unequivocally; Bolton and Gerlt published back-to-back (and cooperative) papers in a recent *Journal of the American Chemical Society* [**106**, 437 and 439 (1984)], while Tsai published somewhat earlier [*ibid.* **105**, 5455 (1983)]. Each investigator used oxygen-17 to label specific phosphorus atoms.

The technique had previously been used in ^{31}P spectrometry of small molecules. When ^{31}P is directly bonded to ^{17}O in such molecules, a phenomenon known as "scalar relaxation of the second kind" occurs because of quadrupolar relaxation of the ^{17}O. As a direct result of this relaxation, the NMR signal for ^{31}P is broadened and becomes less intense. This makes it possible to pick out the signal for the atom to which ^{17}O is attached. But, says Gerlt, "several people have predicted that this kind of relaxation will not occur if the phosphorus-oxygen bond is in a macromolecule."

In fact, the scalar quadrupole relaxation probably does nor occur in most macromolecules. A direct dipolar relaxation unexpectedly does occur, however, with precisely the same result. Bolton and Andrew Joseph used this technique with a complex of polyinosine and polycytidine, for example, to obtain chemical shifts for the ^{31}P nuclei in each polynucleotide. A similar study with a complex of polyadenosine and polyuridine showed no selectivity, however, between spectra in which poly(A) was labeled and in which it was not. This finding, says Bolton, "implies that the residues of both homopolymers may have alternating conformations."

Gerlt, Matthew Petersheim, and Shujaath Mehdi labeled the DNA oligomer CpGpCpG (C, cytidine; G, guanosine) at each of the two outside phosphorus nuclei separately to identify each of the chemical shifts for the molecule. They were then able to use these labeled oligomers in the presence of actinomycin D to show that the drug intercalates between the center guanosine and cytidine units. Tsai and his colleagues were able to use the technique to identify the signal from P_α in the spectrum of adenosine diphosphate bound to arginine kinase. They were also able to demonstrate broadening of ^{31}P peaks in phospholipid bilayers.

This technique, says George Gray of Varian Corporation, "opens up a greater use of ^{31}P spectrometry" because it makes it possible to assign chemical shifts with a relatively small amount of effort. Perhaps the first step, though, is developing a catalog of chemical shifts for phosporus nuclei in various compounds. All three groups are currently directing a good deal of effort to that problem.—THOMAS H. MAUGH II