New turns on old spins

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Quadrupolar cation NMR, particularly of the spin 3/2 sodium and potassium nuclei, has provided one of the more versatile probes of cation domains and motional behavior in living tissue (1). In addition to a fundamental concern to clarify the physical state of intracellular cations, current interest in such studies reflects an awareness that ^{23}Na NMR relaxation behavior may provide an imaging marker for differentiating between normal and diseased or malignant cell types.

A limitation with early in vivo studies was the inability to separate isochronous peaks from intracellular and extracellular $^{23}Na^+$ (or $^{39}K^+$). This problem was largely overcome by the application of anionic shift reagents, which bound and shifted the external signal and allowed the intracellular signal to be observed in isolation (1). However, even though the use of shift reagents may allow the intracellular cation resonance to be studied in isolation, the contribution to this resonance of the various cationic domains remains obscure. The problem faced is how to resolve the contributions from various domains based on intrinsic NMR relaxation properties of the cationic nuclei.

One of the earliest controversies in the field of in vivo cation NMR was over the fraction of intracellular sodium and potassium ions which experience ordered anisotropic environments (2). By now it is clear that the 60% loss of ^{23}Na or ^{39}K NMR intensity that is generally observed in in vivo studies reflects the disappearance from the spectrum of contributions to the NMR signal from the two outer single quantum transitions, each of which contribute 30% to the total integrated intensity. This observation in itself demonstrates that at least some class of bound sodium ions must be motionally restricted, but otherwise provides limited information. At one extreme, the observation could reflect a relatively small class of highly motionally inhibited, anisotropically bound cations in rapid exchange with a larger class of highly mobile cations. For this case, the intensity loss would reflect an unresolved quadrupolar splitting (the static quadrupolar effect). At the other extreme, the intensity loss could reflect a contribution from isotropic motions which are sufficiently rapid to average the quadrupolar splitting to zero, but sufficiently slow that biexponential relaxation occurs (the dynamic quadrupolar effect). Upon Fourier transformation, a bilorentzian curve is observed, for which the broader component (corresponding to the outer transitions) may disappear into the baseline. The consequence of this loss of the broader component is again a 60% decrease in the integrated intensity. In neither extreme is it possible to place significant restraints on the relative populations of cations in various motional domains.

A modern solution to this old problem is to apply pulse sequences which allow multiple quantum coherences to form and evolve. Although in principle such experiments contain no information that is not available from the lineshape, in practice they can provide a clear experimental distinction between dynamic and static quadrupolar effects. The article by Shinar and colleagues (3) in this issue illustrates how such experiments allow this distinction to be made.

In the NMR experiment, only single quantum transitions are directly observable. However, for suitable pulse sequences, multiple quantum coherences can be induced (4, 5). Phase cycling allows these coherences to be selected, and the final observed single quantum coherence will reflect the nature of the intermediate states. In performing such experiments it is said that the nuclear signal is passed through a multiple quantum filter (MQF). MQF signals can be observed for spin 3/2 nuclei in isotropic solution provided that relaxation is biexponential (6, 7). The other situation where spin 3/2 nuclei give MQF signals is in the very slow motion limit where the quadrupolar coupling does not average to zero.

As has been discussed by various groups, double quantum filtration experiments can allow a clear distinction to be made between such dynamic and the static quadrupolar effects (6, 8, 9). This distinction is particularly clear when triple quantum filtration (TQF) and double quantum filtration (DQF) experiments are compared. Shinar and colleagues (3) exploit the approach suggested by Wimperis (5, 9), which involves a DQF experiment with a final excitation pulse of 54.7°. In so doing, they can cleanly resolve contributions due to static and dynamic quadrupolar effects. Shinar and colleagues apply this methodology to a well defined in vivo model system (red blood cells). Some quite interesting results are obtained, which demonstrate that the anisotropy of motion of the sodium ions depends on the integrity of the cytoskeleton network of the red blood cells.

The groundwork underlying experiments such as these is by now well understood. The analysis of intracellular cation resonances will never be trivial, and will only be feasible for carefully controlled experimental systems. Nonetheless, if this note of caution is heeded, then it is anticipated that careful in vivo MQF experiments, such as those described by Shinar and co-workers, will continue to deepen our understanding of the physical state of cations in living cells.

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REFERENCES

1. Springer, C. S., Jr. 1987. Measurement of metal cation compartmentalization in tissue by high-resolution metal cation NMR. *Annu. Rev. Biophys. Biophys. Chem.* 16:375–399.
2. Edzes, H. T., and H. J. C. Berendsen. 1975. The physical state of diffusible ions in cells. *Annu. Rev. Biophys. Bioeng.* 4:265–285.
3. Shinar, H., T. Knubovets, U. Eliav, and G. Navon. 1993. Sodium interaction with ordered structures in mammalian red blood cells detected by Na-23 double quantum NMR. *Biophys. J.* 64.
4. Bax, A., R. Freeman, and S. P. Kempsell. 1980. Natural abundance ^{13}C-^{13}C coupling observed via double-quantum coherence. *J. Am. Chem. Soc.* 102:4849–4851.
5. Bodenhausen, G., H. Kogler, and R. R. Ernst. 1984. Selection of coherence-transfer pathways in NMR pulse experiments. *J. Magn. Reson.* 58:370–388.
6. Jaccard, G., S. Wimperis, and G. Bodenhausen. 1986. Multiple-quantum NMR spectroscopy of S = 3/2 spins in isotropic phase: a new probe for multiexponential relaxation. *J. Chem. Phys.* 85:6282–6293.
7. Pekar, J., and J. S. Leigh. 1986. Detection of biexponential relaxation in sodium-23 facilitated by double-quantum filtering. *J. Magn. Reson.* 69:582–584.
8. Rooney, W. D., T. M. Barbara, and C. S. Springer, Jr. 1988. Two-dimensional double-quantum NMR spectroscopy of isolated spin 3/2 systems: sodium-23 examples. *J. Am. Chem. Soc.* 110:674–681.
9. Eliav, U., H. Shinar, and G. Navon. 1992. The formation of a second-rank tensor in sodium-23 double-quantum-filtered NMR as an indicator for order in a biological tissue. *J. Magn. Reson.* 98:223–229.