Ins and Outs of Biological Tissue Bi-exponential Quadrupolar NMR Signal Relaxation:

There is No Bound Sodium

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Among the most abundant magnetic atomic nuclei in biology, three have the spin quantum number I = 3/2. These are: ^{23}Na, ^{35}Cl, and ^{39}K.[1] In tissue, these elements exist almost exclusively as the mobile mono-aquoions, Na_{aq}^+, Cl_{aq}^-, and K_{aq}^+. Since they are isoelectronic with closed shell inert gas atoms Ne (for Na^+) and Ar (for Cl^- and K^+), they do not form covalent chemical bonds with other atoms. (A small atom fraction of biological Cl atoms, in the Cl^0 oxidation state, forms single covalent bonds with C atoms.) Their homeostatic *tissue* concentrations are: $[Na_t^+] = 44$, $[Cl_t^-] = 56$, and $[K_t^+] = 47$ mM.[1] Most importantly, however, these are not their compartmental concentrations.

The almost exclusive biological role of these species is manifest by their maintenance in homeostatic trans-(cell)membrane concentration gradients. Typical values are: $[Na_i^+] = 15$, $[Na_o^+] = 135$ mM; $[Cl_i^-] = 15$, $[Cl_o^-] = 105$ mM; and $[K_i^+] = 150$, $[K_o^+] = 5$ mM,[2] where the "i" and "o" subscripts signify inside and outside cells, respectively. Taking the typical membrane potential (E_m) to be that caused by homeostatic cellular K^+ efflux, 90 mV (inside negative), the electrochemical potential free energy values are ΔG_{Na} = -14.4 kJ/mole for Na^+ influx, and ΔG_{Cl} = - 3.62 kJ/mol for Cl^- efflux. Continued expenditure of metabolic energy is required to sustain these conditions. Thus, the Na^+ and Cl^- gradients serve to store chemical energy derived from ATP hydrolysis, and that of K^+ maintains the cell membrane potential. These are absolutely vital functions for cell life, and are driven by the continual operation of the crucial plasma membrane Na^+,K^+-ATPase (NKA) enzyme, the "sodium pump." Particularly prominent roles are found in excitatory signal transmission.

The *in vivo* ^{23}Na signal is much larger than those of ^{35}Cl and ^{39}K.[1] And, obviously, the Na^+ biological role is crucial. Thus, it has long been hoped ^{23}Na MRI could prove of clinical benefit. However, realization has been difficult. Current human ^{23}Na MRI voxel volumes are approximately 60 μL. This means there are typically 6 x 10^6 cells in a voxel. At best, quantitative ^{23}Na MRI can assess the averaged, tissue concentration. However, as detailed above, this gives no information on the essential trans-cytolemmal $[Na^+]$ gradient. This is because $[Na_t^+] = v_e[Na_o^+] + v_i[Na_i^+]$, where v_e and v_i are the extra- and intracellular volume fractions, respectively. Clinical changes in $[Na_t^+]$ can be caused by: a) changes in v_e (= 1 – v_i) at constant $[Na_o^+]$, $[Na_i^+]$; b) changes in $[Na_o^+]$, $[Na_i^+]$ (or both) at constant v_e, v_i; or c) some combination of these. This is the *Hilal ambiguity*.[3] Discriminating intra- and extracellular ^{23}Na signals, either from living animals using a shift reagent[4] or from cell suspensions with no shift reagent,[5] does not resolve this confound. These approaches give only the $v_i[Na_i^+]$ and $v_e[Na_o^+]$ products. They cannot lead to ΔG_{Na} mapping, an ultimate goal. ($\Delta G_{Na} = RT\ln\{[Na_i^+]/[Na_o^+]\} + eFE_m$: e is the electron electric change and F the Faraday constant.) The $[Na_i^+]$ and v_i values must be determined independently.

Complicating the situation further is the fact that ^{23}Na is a spin 3/2 nucleus. Even when the latter is isolated (not J-coupled to another spin – the usual case for ^{23}Na), it has four spin states (energy levels).[4,6-9] This means a single ^{23}Na nucleus in any particular Na^+ molecular environment can exhibit one of three single quantum (SQ) resonances. This is dramatically different than the case for a spin ½ nucleus (^{1}H) with only two spin states, and has led to much misinterpretation. The MRI world is accustomed to the fact that a single type of ^1H_2O molecule in a single type of molecular environment gives only a single signal (one site: one resonance).

Fundamentally, an MR resonance has four properties: 1) intensity; 2) Larmor frequency (ν_L); 3) longitudinal relaxation rate constant, $R_1 = 1/T_1$; and 4) transverse relaxation rate constant, $R_2 = 1/T_2$. Even for a single molecular environment, a spin 3/2 signal has its intensity partitioned into three relative values (0.3:0.4:0.3) determined by quantum mechanical principles.[4,6-9] Their resonance frequencies can be different ($\nu_0 + \langle\nu_Q\rangle$:$\nu_0$: $\nu_0 - \langle\nu_Q\rangle$), where $\langle\nu_Q\rangle$ is the temporal mean quadrupolar spectroscopic splitting factor ("coupling constant;" $\langle\nu_Q\rangle = \langle\omega_Q\rangle/2\pi$).[4,6-9] These are usually referred to as one "central" and two "satellite" transitions.

A spin 3/2 nucleus has an *electric* quadrupole. Thus, its orientation, relative to the *magnetic* field ($\mathbf{B}_0$) direction, depends on the orientation (in $\mathbf{B}_0$) of the *electric* field *gradient* (EFG; d**E**/dx; q) of its immediate molecular environment. The nucleus also has a *magnetic* dipole. So, the orientation of the latter in $\mathbf{B}_0$ also depends on that of q. The ^{23}Na

nuclear quadrupole moment (Q) is rather large (10 $(fm)^2$), and when a $^{23}Na^+$ ion is "trapped" in an anisotropic site with a very large q, as is often the case in the solid state, ν_Q for ^{23}Na can approach 10 MHz.[4] The value of ν_Q is proportional to e^2qQ/h, where h is Planck's constant. Thus in the solid state, q can be as great as 10×10^{30} (V/m)/nm.

However, in any aqueous fluidic environment (such as biological tissue), there is much molecular motion. Fluctuation of q magnitude/orientation is characterized by a ν_Q modulation correlation time constant, τ_c. The mean Na_{aq}^+ structure ($Na(OH_2)_6^+$) is isotropic (q = 0). However, because of hydration shell normal mode vibrations, $\tau_c = \tau_v < 1$ ps, $\langle \nu_Q \rangle = 0$. The central and two satellite resonances are isochronous. Upon temporal averaging, the square of ν_Q, $\nu_Q^{rms} = 1$ MHz.[4,7]

Molecular motion also has significant consequences for the third and fourth signal properties, the relaxation rate constants. There are two SQ R's for each: $^{1Q}R_{1s}$ and $^{1Q}R_{1f}$, and $^{1Q}R_{2s}$ and $^{1Q}R_{2f}$, where the s and f subscripts represent "slow" and "fast," respectively. We will not dwell on $^{1Q}R_{1s}$ and $^{1Q}R_{1f}$ here, because their relationships to the spin states are complicated,[7] and they are rarely discriminated.

The behavior of $^{1Q}R_{2s}$ and $^{1Q}R_{2f}$, however, is crucial. Fortunately, these can be identified with the central and satellite resonances, respectively. The very fast modulation in the Na_{aq}^+ environment causes $^{1Q}R_{2f}$ to equal $^{1Q}R_{2s}$. It is too fast for efficient relaxation. Thus, there is a single, narrow resonance with a single $^{1Q}R_2$ value – a *homogeneous* resonance, with a single exponential transverse decay. We have termed this a *type d spectrum*.[4,6-9] It is also often said to be in the "extreme narrowing limit."

The mean lifetime (τ_M) of the sodium-oxygen coordinate covalent bond in Na_{aq}^+ is approximately one nanosecond. During its diffusional excursions in the tissue *milieu*, the Na_{aq}^+ ion encounters other molecules and, sometimes, one inner coordination sphere water molecule is transiently replaced by (almost exclusively) an oxygen atom of the other molecule. This new sodium-oxygen bond provides a significant q, but does not last even a nanosecond. Encountering further molecules gives transient q's with all possible orientations in $\mathbf{B}_0$. Generally, there is no macroscopic orientational order in the surroundings.

Thus, a much slower modulation, 10^3 MHz (τ_M^{-1}), is superimposed on the fast, fundamental 10^6 MHz (τ_v^{-1}) fluctuation of ν_Q.[7] Even if the other molecule is as small as 100 kDa, its rotational correlation time, τ_r, is > 60 ns. So, in the complete expression, $\tau_c^{-1} = \tau_v^{-1} + \tau_M^{-1} + \tau_r^{-1}$, only the first two terms are of any consequence: $(60 \text{ ns})^{-1}$ is only 17 MHz.

Values of ν_L for ^{23}Na MR studies are tens of MHz. If the slow ν_Q modulation (τ_M^{-1}) is near or even less than ω_L ($2\pi\nu_L$), and has sufficient amplitude (significant q values), it acts particularly to catalyze (increase) $^{1Q}R_{2f}$, which is more sensitive to slow fluctuations than is $^{1Q}R_{2s}$.[7] The $^{1Q}R_{2f}$ and $^{1Q}R_{2s}$ values become different: $^{1Q}R_{2f} > {}^{1Q}R_{2s}$. Thus, though the resonances remain isochronous, and the satellite transition remains *homogeneous*, the transverse relaxation becomes bi-exponential. We termed this a *type c spectrum*.[4,6-9]

In biological tissue, the *type c* condition obtains almost exclusively for the ^{23}Na resonance. [More restricted conditions (with some macroscopic orientational order), *type b* and *type a*,[4,6-9] are very rare.] Unfortunately, however, *type c* has been often misinterpreted. Let us re-state the situation: the resonance consists of two signals with the same resonance frequency but two different $^{1Q}R_2$ (T_2) values. Depending on the experimental dead-time, some of the signal with $^{1Q}R_{2f}$ usually decays before acquisition begins ($^{1Q}T_{2f}$ values can be a few hundred μs.[6]). This has been called "^{23}Na MR invisibility," and has often been interpreted that some Na is missing, or "bound." But this is not the case. *All* tissue Na contributes to *each* signal; satellite and central. Some of the signal is missing, not some of the Na. The central transition acts very much like that of a spin ½ nucleus [the $^{1Q}R_{2s}$ expression has no "secular" term].[4,6-9] The resonances of ^{35}Cl and ^{131}Xe (I = 3/2) can exhibit similar spectra,[7] and no one thinks of Cl^- or Xe as forming covalent bonds. This misinterpretation contributed to one of the most significant controversies in bio-science history.[10]

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