POTASSIUM-39 NUCLEAR MAGNETIC RESONANCE

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Summary

Potassium nuclear magnetic resonance is investigated as a tool for studying potassium ion interactions in biochemistry. The potassium line width in 2 M potassium chloride is 7.1 ± 0.6 Hz. The line is substantially broadened in the presence of inorganic phosphate and adenosine triphosphate at pH values greater than 6. It is shown that the potassium-39 line is broadened significantly by the addition of pyruvate kinase thus demonstrating a direct interaction of the metal ion with the enzyme in the absence of added cofactors.

There has been increasing application of nuclear magnetic resonance (nmr) of nuclei other than protons to the investigation of biochemical problems. To date the chlorine-35 has been most successfully used to probe metal-macromolecule interactions[1-3]; however, nuclear magnetic resonance studies using sodium-23, calcium-43, magnesium-25 and thallium-205 have indicated that the nmr signal from these species may also directly answer certain questions about the interactions of these metal ions with small molecules, proteins, membranes and whole tissues.[4-8] Although Deverell and Richards[9] have reported experiments using potassium-39, no work has been reported where the potassium signal was exploited for the investigation of potassium interactions with potassium activated enzymes for example. Potassium is present in relatively high concentrations in most living cells and potassium interactions may be of great importance; however, because it lacks other convenient spectroscopic properties, potassium is a poorly understood cofactor in biochemistry. This note will describe several basic experiments that indicate that potassium nmr may provide useful information about potassium-protein interactions.

Potassium-39 has a natural abundance of 93 percent and like the other alkali metals mentioned, has a nuclear spin of 3/2 and also possesses a sig-

nificant nuclear electric quadrupole moment. The interaction of the nuclear electric quadrupole with the electric environment of the potassium ion provides an efficient and usually dominant relaxation mechanism for the nuclear spin system so that the nuclear spin relaxtion time is given by the equation

$$\Delta\nu = \frac{2\pi}{5} (e^2qQ)^2 \tau_c \qquad (1)$$

where the asymmetry parameter has been neglected, $\Delta\nu$ is the full line width at half height, e the unit electric charge, q the electric field gradient at the nucleus of quadrupole moment Q, and τ_c the correlation time for the reorientation of the electric field gradient with respect to the direction of the applied magnetic field.[10] It is expected that the exchange of potassium ion with the several sites available to it in an aqueous solution containing potential potassium binding sites will be quite rapid.[11] In the limit where the exchange is rapid compared with the line width at each site, the relaxation equation becomes the weighted average of the relaxation times accociated with each site:

$$\Delta\nu = \sum_{\text{sites}} P_i \Delta\nu_i \qquad (2)$$

where P_i is the probability of finding a potassium ion in the solution at a site of type i, and $\Delta\nu_i$ is the full width of the nmr line at the ith site.[12]

For an aqueous solution of potassium chloride it is expected that the electric environment of the potassium ion will be approximately symmetric, the field gradient small and the line width small. Figure 1 shows a representative spectrum of aqueous 2 M potassium chloride recorded on a Varian DP-60 NMR spectrometer equipped with a V-4210A variable frequency r.f. unit and a Princeton Applied Research Model 121 lock-in amplifier. The spectrum shown was recorded using a modulation frequency of 400 Hz with modulation amplitude and phase adjusted to display the first side band of the absorption signal. The r.f. level was below saturation. As shown in curve a, the line width is 7.1 ± 0.6 Hz.

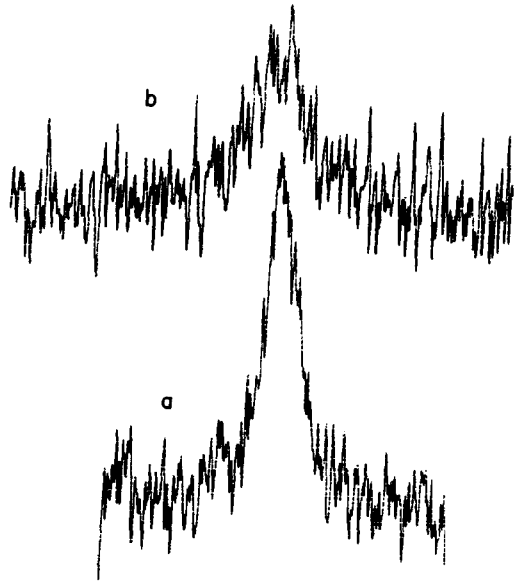

Figure 1. Curve a: Potassium-39 nmr signal obtained from a 2.0 M aqueous potassium chloride solution. Line width is 7.1 ± 0.6 Hz. Curve b: Potassium-39 nmr signal in 2.0 M potassium chloride saturated with pyruvate kinase.

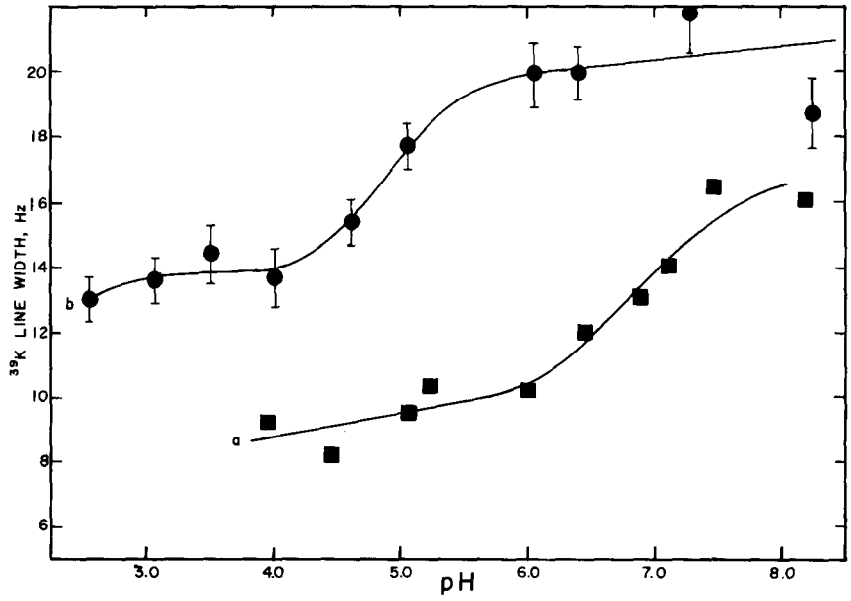

Figure 2. Curve a: Potassium-39 line width as a function of pH for a 2.0 M potassium chloride solution that is 1.0 M in phosphate. Curve b: Potassium-39 line width as a function of pH for a 2.0 M potassium chloride solution that is 0.2 M in adenosine triphosphate.

Figure 2 shows the potassium line width as a function of pH for a 1 M phosphate solution and for 0.2 M ATP solutions in 2 M potassium chloride. In each case the increase in line width with increasing pH may be explained by the exchange of the potassium ion with increasingly ionized phosphate sites in solution. The greatest change in line width occurrs in the region where the pH closely approximates the pK of the acids involved.[13] These simple experiments indicate that potassium nmr may be a useful tool for investigating the potassium interactions with phosphate or other potential potassium binding sites on macromolecules. To test this hypothesis the potassium requiring enzyme pyruvate kinase was chosen because it has been characterized by a variety of methods and direct evidence for the interaction of thallium(I) with the enzyme was obtained by nuclear magnetic resonance.[8]

When pyruvate kinase[14] is added to a 2 M potassium chloride solution at pH 7, a broadened line is observed as shown in figure 1 curve b. The broadening observed on the addition of enzyme may be explained by a direct binding and exchange of the metal ion with the protein. Similar experiments using bovine serum albumin solutions as concentrated as 10^{-3} M caused an increase in line width of less than 3 Hz. This indicates that the contributions due to nonspecific effects such as viscosity are small, and cannot account for the magnitude of the broadening observed on the addition of pyruvate kinase.

The addition of adenosine triphosphate and magnesium to the enzyme solution caused line-width changes on the order of experimental error; however, the addition of manganese ion to the enzyme solutions containing millimolar ATP and magnesium ion caused the line width to decrease by 30 percent. The earlier results obtained by thallium(I) nmr indicated that the manganese ion and thallium(I) ion occupied positions on the enzyme that were very close together. While this is consistent with the present potassium results, the immediate proximity of the two ions is not a requirement.

This series of experiments demonstrate that potassium-39 nmr may be used directly to investigate potassium-enzyme interaction. This approach to

studying the alkali and alkaline earth interactions promises to be very fruitful because the method does not require optical transparency, is non-destrutive and contains kinetic as well as structural information.

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