A Multinuclear Nuclear Magnetic Resonance Study of the Monovalent–Divalent Cation Sites of Pyruvate Kinase†

Frank M. Raushel‡ and Joseph J. Villafranca*

ABSTRACT: The effectiveness of measuring distances between monovalent and divalent cation sites on enzymes has been examined by ^{6}Li, ^{7}Li, ^{14}N, ^{15}N, ^{23}Na, ^{39}K, ^{85}Rb, ^{87}Rb, and ^{133}Cs nuclear magnetic resonance (NMR). Measurements were made of the paramagnetic effect of enzyme-bound Mn^{2+} on the longitudinal spin–lattice relaxation rate ($1/T_1$) of the monovalent cations by using Mn^{2+} at the divalent cation site of pyruvate kinase. Distances from Mn^{2+} to the monovalent cations in the enzyme–Mn^{2+}–M^+ complex are as follows: $^{6}Li^+$, 8.5 Å; $^{7}Li^+$, 8.4 Å; $^{15}NH_5^+$, 7.0 Å; $^{133}Cs^+$, 7.7 Å. The measured distances in the enzyme–Mn^{2+}–M^+–phosphoenolpyruvate (PEP) complex are as follows: $^{6}Li^+$, 5.7 Å; $^{7}Li^+$, 5.7 Å; $^{14}NH_4^+$, 4.4 Å; $^{15}NH_4^+$, 4.4 Å; $^{133}Cs^+$, 6.0 Å. In the complex with PEP, a lower limit distance could be placed on Mn^{2+} to $^{23}Na^+$ (≥4.5 Å), $^{39}K^+$ (≥3.7 Å), and $^{87}Rb^+$ (≥4.1 Å). These results show a 2–3-Å reduction in the distance between Mn^{2+} and the monovalent cation upon addition of PEP to the enzyme. Additionally, the Mn^{2+} to monovalent cation distances in the enzyme complexes with PEP correlate very well with the observed levels of activation of pyruvate kinase by these ions. In this study we used a novel method to determine the correlation time (τ_c) for the dipolar Mn^{2+}–M^+ interaction. From the ratio of the paramagnetic effects of enzyme–Mn^{2+} on the $1/T_1$ values of the two isotopes of Li^+, NH_4^+, or Rb^+, a unique value of τ_c is obtained at a single magnetic field strength. Further analysis of this method reveals that for $^{6}Li^+$, $^{7}Li^+$, $^{15}NH_4^+$, and $^{133}Cs^+$, distances from enzyme–Mn^{2+} to these monovalent cations can be reliably measured up to 12–20 Å, while for $^{39}K^+$, $^{85}Rb^+$, and $^{87}Rb^+$ the practical upper limit is ~4 Å. For $^{14}NH_4^+$, $^{205}Tl^+$, and $^{23}Na^+$, Mn^{2+} to M^+ distances could be measured in the 5–8-Å range. Thus, the multinuclear NMR study of the monovalent cation site of pyruvate kinase reported herein has revealed the feasibility of using NMR to relate structure to function in the large class of enzymes that are activated by monovalent cations.

A large number of enzymes display an absolute requirement for the addition of a monovalent cation for maximal activity (Suelter, 1970). The most studied enzyme of this group is pyruvate kinase which shows a wide range of maximal activities depending on the monovalent cation used to activate the enzyme (Kayne, 1973). For example, lithium activates only 2% as well as does potassium. The other monovalent cations are of intermediate activity (Kayne, 1973). It has been observed that the degree of activation of pyruvate kinase by the various monovalent cations appears to correlate with the crystalline ionic radius of these cations. The amount of activation is found to decrease as the ionic radius increases or decreases relative to that for K^+ (Kachmar & Boyer, 1953). However, the precise function of these monovalent cations in catalysis is not known.

In an attempt to determine the exact location of the monovalent cation site relative to the sites for the other ligands of pyruvate kinase, a number of laboratories have undertaken measurements of the distance from enzyme-bound Mn^{2+} to the monovalent cation site by using nuclear magnetic resonance (NMR).[1] Reuben & Kayne (1971) have reported distances of 4.9 and 8.2 Å between $^{205}Tl^+$ and Mn^{2+} in the enzyme–Mn^{2+}–Tl^+–phosphoenolpyruvate (PEP) complex and the enzyme–Mn^{2+}–Tl^+ complex, respectively. In a ^{7}Li NMR study, Hutton et al. (1977) reported distances of 5.8 and 11.0 Å for these enzyme–Mn^{2+} complexes with $^{7}Li^+$. Since Li^+ activates only 3% as well as Tl^+, these authors proposed that the longer metal–metal distance for the Li^+ complexes compared with the Tl^+ complexes correlated with the large decrease in enzymatic activity. Ash et al. (1978) pointed out that there are two interconvertible forms of the enzyme–Mn^{2+}–Li^+–PEP complex (Reed & Cohn, 1973) that Hutton et al. (1977) did not take account of in their data analysis. Repeating the ^{7}Li NMR experiments, Ash et al. (1978) obtained distances between Mn^{2+} and Li^+ that are nearly identical with the Mn^{2+}–Tl^+ distances of Reuben & Kayne (1971). However, the reason for the difference in the measured distances in these two ^{7}Li NMR studies is in the choice of a correlation time (τ_c) for the dipolar Mn^{2+}–Li^+ interaction. Hutton et al. (1977) used a value of 9.4 ns while Ash et al. (1978) used a value of 1.7 ns. These apparently disparate correlation times were obtained from a study of water proton relaxation rates. Clarification of this discrepancy is needed.

In this paper we report an extended study of the monovalent and divalent cation sites of pyruvate kinase which includes most of the monovalent cations that activate pyruvate kinase. The ions investigated were $^{6}Li^+$, $^{7}Li^+$, $^{14}NH_4^+$, $^{15}NH_4^+$, $^{23}Na^+$, $^{39}K^+$, $^{85}Rb^+$, $^{87}Rb^+$, and $^{133}Cs^+$. Although most of these isotopes are quadrupolar and some have very fast relaxation rates, we previously pointed out that a number of them are particularly attractive for study by NMR (Villafranca & Raushel, 1980). Additionally, we developed a novel method for unambiguously determining the correlation time for the dipolar interaction of a paramagnetic center with monovalent cations (Raushel & Villafranca, 1980). This method is based on determining the ratio of spin–lattice relaxation rates of the two isotopes of Li^+, NH_4^+, or Rb^+ interacting with an enzyme-bound paramagnetic center, e.g., Mn^{2+}. The calculated

† From the Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802. *Received April 8, 1980.* Supported by the National Science Foundation (PCM-7807845). Support for the nuclear magnetic resonance instrumentation from the National Science Foundation is also gratefully acknowledged.

* Correspondence should be addressed to this author. J.J.V. is an Established Investigator of the American Heart Association.

‡ F.M.R. is a National Research Service awardee (AM-05996). Present address: Department of Chemistry, Texas A&M University, College Station, TX 77843.

[1] Abbreviations used: NMR, nuclear magnetic resonance; PEP, phosphoenolpyruvate; EDTA, ethylenediaminetetraacetic acid; EPR, electron paramagnetic resonance.

0006-2960/80/0419-5481$01.00/0 © 1980 American Chemical Society

Table I: Relaxation Times of Monovalent Cations in Various Solutions[a]

nucleus	frequency (MHz)	relaxation times (s)				
		Tris[b]	Mn^{2+}, Tris[c]	Mn^{2+}-enzyme[d]	Mn^{2+}-PEP-enzyme[e]	Mg-PEP-enzyme[f]
$^{6}Li^{+}$	29	151	96	17	2.0	93
$^{7}Li^{+}$	78	15.1	11.9	4.4	0.79	10.8
$^{7}Li^{+}$	39	20.6	14.5	3.3	0.45	13.4
$^{14}NH_4^{+}$	14	0.51	0.46	0.41	0.32	0.52
$^{15}NH_4^{+}$	10	42	26	6.8	0.53	41
$^{23}Na^{+}$	53	0.043	0.045	0.041	0.040	0.041
$^{23}Na^{+}$[g]	53	0.043	0.044	0.040	0.040	0.041
$^{39}K^{+}$	9	0.042	0.041		0.028	0.028
$^{39}K^{+}$[h]	9	0.037	0.037		0.018	0.022
$^{85}Rb^{+}$	19	0.0019	0.0019			
$^{87}Rb^{+}$	65	0.0018	0.0017		0.0017	0.0017
$^{87}Rb^{+}$[i]	65	0.0018	0.0018		0.0016	0.0016
$^{133}Cs^{+}$	26	10.3	9.8	4.5	2.1	7.4

[a] All solutions are 50 mM tris(hydroxymethyl)aminomethane (Tris), pH 7.5, 20% D_2O, and 100 mM in monovalent cation except as noted below. Precision in T_1 measurement is ±5%. [b] Buffer only. [c] 200 μM $MnCl_2$. [d] 200 μM $MnCl_2$ and 40 μM pyruvate kinase sites. [e] 200 μM $MnCl_2$, 40 μM pyruvate kinase sites, and 1.0 mM PEP. [f] 2.4 mM $MgCl_2$, 40 μM pyruvate kinase sites, 1.0 mM PEP, and 1.0 mM EDTA. [g] 25 mM NaCl. [h] 20 mM KCl and 80 μM enzyme sites when added. [i] 20 mM RbCl and 80 μM enzyme sites when added.

distances between the monovalent and divalent cation sites of pyruvate kinase determined in this report correlate well with the degree of activation exhibited by the various monovalent cations.

Materials and Methods

Materials. Rabbit muscle pyruvate kinase was obtained from Sigma Chemical Co. as a lyophilized salt-free powder. The specific activity of this preparation (Lot 29C-9790) was 130 units/mg. The concentration of active enzyme was calculated from the specific activity of 250 units/mg for fully active enzyme (Boyer, 1962). $^{15}NH_4Cl$ (95 atom %) was supplied by Merck, and $^{6}LiOH$ (95 atom %) was from Oak Ridge National Laboratory. RbCl and CsCl (ultrapure) were obtained from Alfa Inorganics.

Nuclear Magnetic Resonance Measurements. Spin–lattice relaxation times (T_1) for the various monovalent cations were determined by using a 180°–τ–90° pulse sequence with a Brüker WP-200 multinuclear NMR spectrometer. A few experiments with $^{15}NH_4^{+}$ and $^{7}Li^{+}$ were also performed with a JEOL PS-100 NMR spectrometer. The T_1 values and their standard errors were calculated by using a computer program supplied by the Nicolet Instrument Corp. The T_1 measurements were determined at 30 °C with 1.25-mL samples in 10-mm NMR tubes containing 20% D_2O, 50 mM tris(hydroxymethyl)aminomethane (Tris), pH 7.5, and various other additions as indicated in the text and in Table I. Delay times of at least $5T_1$ were used in all experiments. Usually a single pulse was used to obtain a spectrum at each τ value for $^{6}Li^{+}$, $^{7}Li^{+}$, $^{15}NH_4^{+}$, $^{23}Na^{+}$, and $^{133}Cs^{+}$. Ten to one thousand scans were accumulated for each τ value for T_1 measurements of $^{39}K^{+}$, $^{85}Rb^{+}$, $^{87}Rb^{+}$, and $^{14}NH_4^{+}$. Broad-band proton decoupling was used in the measurement of $^{6}Li^{+}$ to take advantage of the nuclear Overhauser enhancement of 2.7 (Wehrli, 1976).

Mn^{2+}–Monovalent Cation Distance Calculations. The distances between enzyme-bound Mn^{2+} and the various monovalent cations were determined by measuring the effect of the appropriate enzyme–Mn^{2+} complex on the spin–lattice relaxation times of those ions. The Solomon–Bloembergen equation (Solomon & Bloembergen, 1956) was used to compute the distances.

$$r = C[T_{1M}f(\tau_c)]^{1/6} \quad (1)$$

In this equation r is the distance between the two sites (in angstroms), T_{1M} is the paramagnetic contribution of the enzyme–Mn^{2+} complex to the spin–lattice relaxation rate of the monovalent cation, and $C = [(2/15)\gamma_I^2 g^2 \beta^2 S(S+1)]^{1/6}$ where γ_I is the magnetogyric ratio, S is the electron spin, and β is the Bohr magneton. The values of C for the Mn^{2+} interaction with ^{6}Li, ^{7}Li, ^{14}N, ^{15}N, ^{23}Na, ^{39}K, ^{85}Rb, ^{87}Rb, and ^{133}Cs are 429, 593, 338, 379, 521, 292, 372, 560, and 413, respectively. $f(\tau_c)$ is calculated by

$$f(\tau_c) = \frac{3\tau_c}{1 + \omega_I^2\tau_c^2} \quad (2)$$

where ω_I is the nuclear Larmor precession frequency and τ_c is the correlation time for the dipolar interaction. The paramagnetic contribution ($1/T_{1M}$) of the enzyme–Mn^{2+} complex to the observed relaxation rate is calculated by

$$p\left(\frac{1}{T_{1M}}\right) = \frac{1}{T_{1(E\cdot Mn)}} - \frac{1}{T_{1(E\cdot Mg)}} - \frac{1}{T_{1Mn}} \quad (3)$$

where $1/T_{1(E\cdot Mn)}$ and $1/T_{1(E\cdot Mg)}$ are the observed relaxation rates in the presence of enzyme–Mn^{2+} and enzyme–Mg^{2+}, respectively, and p is the mole fraction of monovalent cation bound to the enzyme–Mn^{2+} complex. $1/T_{1Mn}$ is the contribution due to free Mn^{2+} in solution and is calculated from the difference in relaxation rates between samples of monovalent cations in buffer with and without Mn^{2+} in the absence of enzyme.

Results and Discussion

Effect on Mn^{2+}–Pyruvate Kinase on Spin–Lattice Relaxation Rates of Monovalent Cations. The values for the spin–lattice relaxation times of the various monovalent cations in the presence of different solutions are shown in Table I. All experiments were performed at 30 °C since Ash et al. (1978) have shown by electron paramagnetic resonance that the pyruvate kinase–Mn^{2+}–Li^{+}–PEP complex can exist in two conformations. The conformation most resembling the "K^{+}-like" spectrum greatly predominates at higher temperatures. In the control samples with Mg^{2+}, EDTA was added to remove any bound paramagnetic impurities that may have been introduced with the enzyme. As can be seen from Table I, substantial effects on the spin–lattice relaxation times of $^{6}Li^{+}$, $^{7}Li^{+}$, $^{14}NH_4^{+}$, $^{15}NH_4^{+}$, and $^{133}Cs^{+}$ are caused by the addition of Mn^{2+}–pyruvate kinase to 100 mM solutions of these ions. In agreement with the results of Reuben & Kayne (1971), Ash et al. (1978), and Hutton et al. (1977), there is a substantially larger effect on the T_1 values in the enzyme–Mn^{2+}–M^{+}–PEP

Table II: Calculated Mn^{2+} to Monovalent Cation Distances in Complexes of Pyruvate Kinase (47 kG)

nucleus	act.[a]	radius[b] (Å)	$1/T_{1M}$[c] (s)	$1/T_{1M}$[d] (s)	r[e] (Å)	r[f] (Å)
$^{6}Li^{+}$	2	0.68	128	1340	8.5 ± 0.4	5.7 ± 0.3
$^{7}Li^{+}$	2	0.68	324	3210	8.4 ± 0.4	5.7 ± 0.3
$^{14}NH_4^{+}$	81	1.43		2140		4.4 ± 0.3
$^{15}NH_4^{+}$	81	1.43	271	4620	7.0 ± 0.4	4.4 ± 0.3
$^{23}Na^{+}$	8	0.97		≤10000		≥4.5
$^{39}K^{+}$	100	1.33		≤2500		≥3.7
$^{87}Rb^{+}$	65	1.47		≤20000		≥4.1
$^{133}Cs^{+}$	9	1.67	205	840	7.7 ± 0.4	6.0 ± 0.3
$^{205}Tl^{+}$	61	1.47	3000	63500	8.0 ± 0.4	4.8 ± 0.3

[a] Kinetic activity relative to that for K^+ (Kayne, 1973). [b] Crystalline ionic radii of monovalent cations (Weast, 1969). [c] Paramagnetic contribution to the relaxation rate in the enzyme-Mn^{2+}-M^+ complex. [d] Paramagnetic contribution to the relaxation rate in the enzyme-Mn^{2+}-M^+-PEP complex. [e] Distance between Mn^{2+} and the monovalent cation in the enzyme-Mn^{2+}-M^+ complex. [f] Distance between Mn^{2+} and the monovalent cation in the enzyme-Mn^{2+}-M^+-PEP complex.

complex than in the enzyme-Mn^{2+}-M^+ complex. This is consistent with PEP causing the Mn^{2+}-M^+ distance to shorten significantly. This same phenomenon was also found by Nowak (1973) using monomethylammonium ion as a probe of the monovalent cation site of pyruvate kinase.

Calculation of τ_c. In order to use the data in Table I and the Solomon-Bloembergen equation to calculate internuclear distances, we must first determine a value for the correlation time (τ_c). This correlation time can, in principle, be evaluated from (1) the ratio of T_{1M} and T_{2M}, (2) a frequency dependence of T_{1M}, (3) a frequency and temperature dependence study of the relaxation rates of solvent water, and, (4) line width measurements of enzyme-bound Mn^{2+} using electron paramagnetic resonance. As pointed out by Dwek (1973), there are significant problems associated with each method. For example, with the T_{1M}/T_{2M} ratio method errors can be introduced when there is a substantial contribution to the transverse relaxation rate due to electron-nuclear hyperfine interactions. This has been suggested as the reason for the large T_{1M}/T_{2M} ratio in the ^{205}Tl NMR study by Reuben & Kayne (1971). This results in correlation times that are too large. The correlation time, τ_c, may be dominated by electron-spin relaxation (τ_s) in which case there may be a significant magnetic field dependence of τ_c.

These same arguments can also be applied to τ_c values calculated from water proton relaxation rate studies. The value of τ_c may be different for the protons of H_2O and the nucleus of interest because of significantly different contributions to τ_c by the rotational correlation time (τ_r) and the ligand exchange lifetime, τ_M. The three previously mentioned papers on monovalent-divalent cation distances of pyruvate kinase all used τ_c values estimated from solvent water relaxation rates.

In the monovalent cation series there are three sets of cations which can be studied with either of two isotopes. These are Li^+, NH_4^+, and Rb^+. From the Solomon-Bloembergen equation it is evident that an unambiguous value of τ_c at one magnetic field strength may be calculated if T_{1M} is determined for both isotopes of one of these cations and prevalence of rapid exchange conditions is assumed (Reuben, 1975). The internuclear distance, r, and τ_c are of necessity the same for both isotopes of one cation at one magnetic field strength. Once the value of T_{1M} for each isotope is determined, the value for τ_c is uniquely determined since C and ω_I are known. Shown in Figure 1 is a graph illustrating the variation in τ_c with the ratio of T_{1M} for the two isotopes of Li^+, NH_4^+, and Rb^+. This range of τ_c values (10^{-9}–10^{-8} s) is that usually encountered in enzyme-Mn^{2+} systems (Dwek, 1973). T_{1M} ratios significantly greater than one are predicted for τ_c values ≤ 5 ns with these three isotopic pairs. Thus, this method for obtaining τ_c values is quite useful for enzyme-Mn^{2+} systems as we have

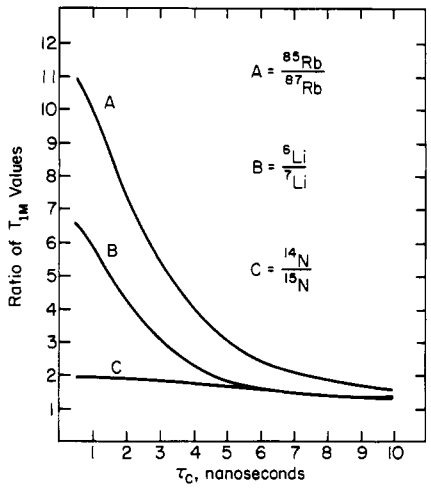

FIGURE 1: Determination of the correlation time (τ) for the dipolar interaction of enzyme-Mn^{2+} from the ratio of T_{1M} for the isotopes of Li^+, NH_4^+, and Rb^+.

previously reported (Raushel & Villafranca, 1980).

In Figure 1 the large ratios of T_{1M} at short correlation times arise from the ratios of the squares of the magnetogyric ratios. At longer correlation times the product of ω_I and τ_c (in eq 2) becomes larger than unity and thus the ratios of T_{1M} level out at unity. The two isotopes of Tl^+ cannot be used with this technique because the magnetogyric ratio is nearly identical for both isotopes. This results in a maximum ratio of T_{1M} values for $^{203}Tl^+$ and $^{205}Tl^+$ of only 1.02.

For our experiments we have measured τ_c in three ways: (1) the ratio of T_{1M} values for two isotopes of the same cation, (2) the frequency dependence of T_{1M} for one cation, and (3) the frequency dependence of T_{1M} for two isotopes of the same cation. The $1/T_{1M}$ values for the PEP-Mn^{2+}-enzyme complexes of $^{6}Li^+$ and $^{7}Li^+$ are 1.34×10^3 and 3.21×10^3 s^{-1}, respectively (Table II). From this ratio and eq 1, a τ_c of 3.7 $\times 10^{-9}$ s is calculated. By use of the relaxation data for $^{7}Li^+$ obtained at 78 and 39 MHz (Table I) and the data of Hutton et al. (1977) at 24 MHz, a τ_c value of 3.4×10^{-9} s was obtained from the ratio of the slope to the intercept in a plot of T_{1M} vs. ω_I^2 (Dwek, 1973). Also, the data for $^{14}NH_4^+$ (at 47 kG) and $^{15}NH_4^+$ (at 23 kG) were used to calculate a τ_c value of 4.7×10^{-9} s from eq 1 and the ratio of T_{1M} values (Table II). The three determinations give quite similar τ_c values with the average value being $(3.9 \pm 0.5) \times 10^{-9}$ s.

Mn^{2+}-Monovalent Cation Distances. Listed in Table II are the $1/T_{1M}$ values obtained from the data in Table I and the distances as calculated from the Solomon-Bloembergen equation. The correlation time determined above (3.9×10^{-9} s) was used in all calculations. The ionic radius and maximal

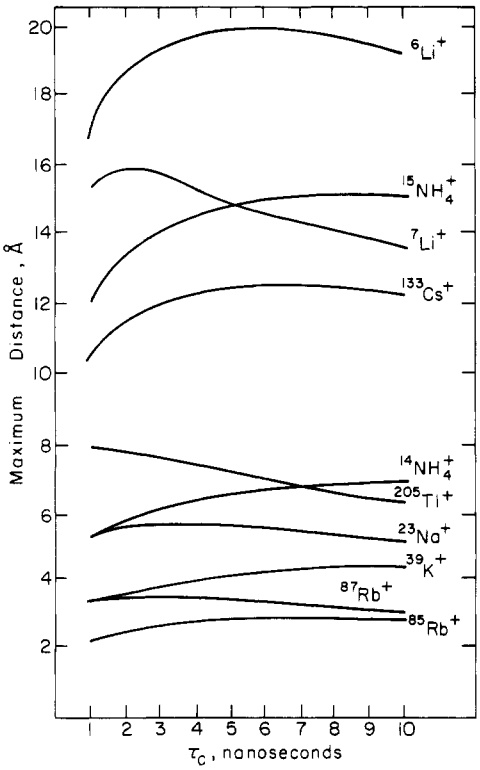

FIGURE 2: Effect of the correlation time (τ_c) on the "maximum" distance between Mn^{2+} and the various monovalent cations that can be measured by using the Solomon–Bloembergen equation. Magnetic field strength is 47 kG. Other conditions are listed in the text.

velocity displayed by each monovalent cation are also listed. The error limits were calculated from a 20% error in $1/T_{1M}$ values and a 15% error in the correlation time. Also included in Table II are the $1/T_{1M}$ values obtained by Reuben & Kayne (1971) for $^{205}Tl^+$ along with the recomputed metal–metal distances calculated by using the correlation time determined from our current data analysis.[2]

At a monovalent cation concentration of 100 mM, site saturation was assumed for all of the monovalent cations except Li^+, since this is the optimum level of monovalent cation for activation as determined kinetically (Kayne, 1973). For Li^+ a dissociation constant of 11 mM was used (Hutton et al., 1977). The assumption of site saturation may not be valid for the Mn^{2+}–M^+–enzyme complexes since there is little information on the binding of monovalent cations to enzyme–Mn^{2+}. If this assumption is not valid, then the distances between the monovalent cation and Mn^{2+} listed in Table II for the Mn^{2+}–M^+–enzyme complex must be viewed as upper limits.

Reliable distances between Mn^{2+} and the monovalent cations were obtained from the present data for Li^+, NH_4^+, and Cs^+, while only lower limits for the distances to Na^+, K^+, and Rb^+ could be obtained. There are two general observations to be made from the data in Table II. The first is that there is a 2–3-Å change in the distance between Mn^{2+} and all of the monovalent cations when PEP is added to the system. This is consistent with the suggestion by Nowak & Mildvan (1972) that the free carboxyl group of PEP is coordinated by enzyme-bound K^+. Their model, based on NMR measurements,

[2] The recomputed metal–metal distances for $^{205}Tl^+$ calculated by using $\tau_c = 3.9 \times 10^{-9}$ s are essentially the same as those reported by Reuben & Kayne (1971) (with $\tau_c = 9.4 \times 10^{-9}$ s). This is because the values for $f(\tau_c)$ (eq 2) are approximately equal for both values of τ_c at a frequency of 24.3 MHz.

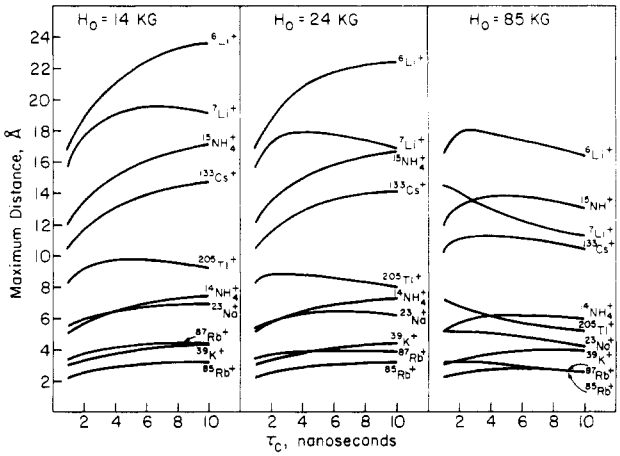

FIGURE 3: Effect of the correlation time (τ_c) on the "maximum" distance between Mn^{2+} and various monovalent cations plotted for three field strengths. (Left) 14 kG; (middle) 24 kG; (right) 85 kG.

predicts a Mn^{2+} to monovalent cation distance of 4.5–6.0 Å. This is precisely the range found for all of the monovalent cations in this report. The second observation is that the cations that are better activators of the enzyme are also significantly closer to the Mn^{2+} in the complex with PEP than are the poorer activators. NH_4^+ and Tl^+ are 4.4–4.8 Å from Mn^{2+} while Li^+ and Cs^+ are 5.7–6.0 Å from Mn^{2+}. This suggests that the orientation of ligands on pyruvate kinase in the presence of the good monovalent activators is significantly different than it is in the presence of the poor monovalent cation activators. There is no obvious trend in the distances in the complexes without PEP, although the distance with $^{15}NH_4^+$ is the shortest measured for the enzyme–Mn^{2+}–M^+ complex.

The Mn^{2+} to $^7Li^+$ distance in the Mn^{2+}–Li^+–PEP–enzyme complex reported in this paper compares favorably with the data of Hutton et al. (1977) and Ash et al. (1978) if their distances are recomputed by using a correlation time of 3.9 $\times 10^{-9}$ s. The data of Hutton et al. (1977) give a distance of 5.7 Å and those of Ash et al. (1978) give a distance of 5.4 Å if their correction for the interconvertible forms of the Mn^{2+}–Li^+–PEP–enzyme complex is not used.[3]

Utility of Monovalent Cations in Distance Determinations. In this report we have confirmed that significant contributions to the T_1 relaxation rates of monovalent cations can be caused by enzyme-bound Mn^{2+}. With this NMR technique one can evaluate the practical upper limits for determining Mn^{2+} to

[3] Ash et al. (1978) have shown by EPR that only 81 ± 5% of the Mn^{2+}–Li^+–PEP–enzyme complex is in a "K^+-like" anisotropic form at 30 °C and 61 ± 5% is in this form at 5 °C. The rest of the enzyme complex at each temperature is in an isotropic form. Using this distribution of enzyme forms and the difference in the observed values of $1/T_{1M}$ at 5 and 30 °C, they calculated a corrected value of $1/T_{1M}$ for both the isotropic and anisotropic forms of the enzyme complex by the simultaneous solution of two equations. They concluded from this analysis that the isotropic form contributes <1% to the observed value of $1/T_{1M}$ at 30 °C. Therefore, the corrected value of $1/T_M$ for the anisotropic form at 30 °C is 19% larger than the observed value. There is, however, a large error associated with this correction since the observed $1/T_{1M}$ values at 5 and 30 °C differ by only 20% and the precision of each T_1 measurement is stated as ±10% (the actual error was much less than this). Because of the large possible error in the actual size of the correction for the observed $1/T_{1M}$, we have elected not to correct any of our Li^+ data for this observation. For this correction it must also be assumed that there is no temperature dependence to τ_s between 5 and 30 °C. Even if we correct our Li^+ data by the largest possible amount (19%), our distance between Li^+ and Mn^{2+} decreases by only 0.2 Å. This is still at least 1.0 Å longer than the distance between Mn^{2+} and NH_4^+ in the Mn^{2+}–NH_4^+–PEP–enzyme complex.

M^+ distances on an enzyme for each nucleus. For the 10 monovalent cations examined, the following assumptions were made: (1) the experiments were done at a magnetic field strength of 47 kG, (2) a decrease in the initial T_1 of 33% could easily be observed, (3) the initial T_1 values were those that are listed in Table I, and (4) the relaxing metal ion was Mn^{2+} and the ratio of free monovalent cation to enzyme-bound monovalent cation was 250.

The distances calculated for this analysis are plotted in Figure 2 as a function of the correlation time for the dipolar interaction. The "maximum" distance that can be measured by using monovalent cations with this relaxation technique is dependent on (1) the magnetogyric ratio, (2) the magnetic field strength, and (3) the relaxation time in the diamagnetic system. Longer distances can be measured for those isotopes with larger magnetogyric ratios and longer diamagnetic spin–lattice relaxation times. Longer distances can also be measured by decreasing the magnetic field. A plot of the maximum internuclear distance as a function of the correlation time at magnetic field strengths of 14, 24, and 85 kG is shown in Figure 3.

The monovalent cations fall into roughly three groups in evaluating the "sensitivity" of this method. $^7Li^+$, $^6Li^+$, $^{15}NH_4^+$, and $^{133}Cs^+$ can be used to measure distances up to 12–20 Å. The "sensitivity" with $^6Li^+$ could possibly be extended to the measurement of even longer distances if experiments are done entirely in D_2O since the T_1 of $^6Li^+$ increases to almost 700 s in 100% D_2O (Wehrli, 1976). $^{14}NH_4^+$, $^{205}Tl^+$, and $^{23}Na^+$ are in the intermediate group and can be used to measure Mn^{2+} to M^+ distances of 5–8 Å. $^{39}K^+$, $^{85}Rb^+$, and $^{87}Rb^+$ are much less "sensitive" than the rest of the monovalent cations although it may be possible to observe paramagnetic effects on the spin–lattice relaxation rate of $^{39}K^+$ in special cases. Rubidium is almost entirely useless for these types of experiments.

Unfortunately, K^+, the cation of most biochemical interest, is not very suitable for study in these systems since it has a low magnetogyric ratio and a very fast spin–lattice relaxation rate. Therefore, the monovalent cation that should receive the most attention in the enzymes activated by monovalent cations is NH_4^+. NH_4^+ has been shown to be almost as effective as potassium in activating most enzymes that require monovalent cations, and we have shown by our calculations (Figure 2) that Mn^{2+} to $^{15}NH_4^+$ distances of up to 15 Å can be measured. In addition to this, paramagnetic effects on $1/T_1$ of $^{14}NH_4^+$ and $^{15}NH_4^+$ can be measured at one field strength in a multinuclear NMR spectrometer for a determination of the correlation time by the methods outlined in this paper and in a previous paper (Raushel & Villafranca, 1980).

References

Ash, D. E., Kayne, F. J., & Reed, G. H. (1978) *Arch. Biochem. Biophys. 190*, 571.

Boyer, P. D. (1962) *Enzymes, 2nd Ed. 6*, 95–113.

Dwek, R. A. (1973) in *Nuclear Magnetic Resonance in Biochemistry*, Clarendon Press, Oxford.

Hutton, W. E., Stephens, E. E., & Grisham, C. M. (1977) *Arch. Biochem. Biophys. 184*, 166.

Kachmar, J. F., & Boyer, P. D. (1953) *J. Biol. Chem. 200*, 669.

Kayne, F. J. (1973) *Enzymes, 3rd Ed. 8*, 353–382.

Nowak, T. (1973) *J. Biol. Chem. 248*, 7191.

Nowak, T., & Mildvan, A. S. (1972) *Biochemistry 11*, 2819.

Raushel, F. M., & Villafranca, J. J. (1980) *J. Am. Chem. Soc. 102*, 6618.

Reed, G. H., & Cohn, M. (1973) *J. Biol. Chem. 248*, 6436.

Reuben, J. (1975) *J. Chem. Phys. 63*, 5063.

Reuben, J., & Kayne, F. J. (1971) *J. Biol. Chem. 246*, 622.

Solomon, I., & Bloembergen, N. (1956) *J. Chem. Phys. 25*, 261.

Suelter, C. H. (1970) *Science 168*, 789.

Villafranca, J. J., & Raushel, F. M. (1980) *Annu. Rev. Biophys. Bioeng. 9*, 363.

Weast, R. C., Ed. (1969) *Handbook of Chemistry and Physics*, 50th ed., Chemical Rubber Co., Cleveland, OH.

Wehrli, F. W. (1976) *J. Magn. Reson. 23*, 527.