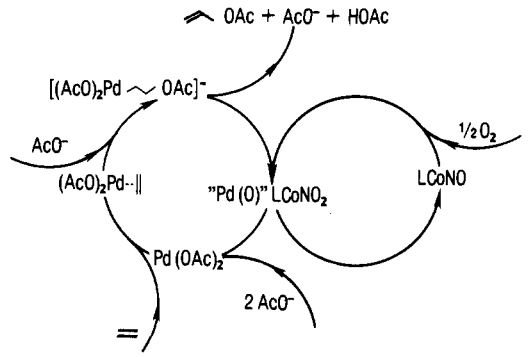
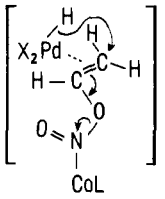
Scheme II



inhibits the ability of palladium(II) to coordinate olefins and to assist in the reoxidation of cobalt–nitrosyl complexes.[9] Acetaldehyde formation from intermediate V likely proceeds via β-hydride elimination followed by hydride shift and oxidation involving intermediate VI.



VI

Cobalt–Nitro Complexes as Palladium Oxidants. Cobalt–nitro complexes I and II are very weak nucleophiles. Therefore, the presence of almost any other competing nucleophile in the above system should result in Wacker-type chemistry. Thus, in acetic acid (Table I, run 7), vinyl acetate is the sole product when ethylene is oxidized by a mixture of cobalt–nitro complex and palladium(II) acetate in an *oxygen-free* atmosphere and *deoxygenated* acetic acid. Importantly, the yield of vinyl acetate is 100% based on the total of $Pd(OAc)_2$ and I. Again, the nitro complex I is cleanly reduced to the corresponding nitrosyl complex Ia. Furthermore, passing oxygen and ethylene into an acetic acid solution of $Pd(OAc)_2$ with either I or II results in the catalytic formation of vinyl acetate. In the catalytic oxidation, the rate of vinyl acetate formation is linear with time, and no metal deposition is apparent. In the absence of I or II, the oxidation is not catalytic, and palladium metal precipitates. These observations clearly underscore the ability of cobalt–nitro complexes to function as efficient reoxidants of palladium(0).[10] Thus, in the presence of nucleophiles, the cobalt–nitro complexes play a similar role to copper(II) in the classical Wacker process (Scheme II).

Conclusion. We have demonstrated a novel model of catalytic olefin oxidation by molecular oxygen. It involves oxygen transfer from the nitro ligand of cobalt–nitro complexes to palladium(II)-bound olefins followed by reoxidation of the reduced nitrosyl ligand by molecular oxygen. The palladium *remains* in the divalent state throughout the catalytic cycle and serves exclusively as a cocatalyst. Our future effort will be directed toward modifying the system to produce glycol derivatives or possibly epoxides.

(9) The cobalt–nitrosyl complexes cannot be reoxidized in the absence of a base or palladium(II). The role of Pd(II) is not fully understood at this time, but it may be analogous to the role of Lewis acids; see ref 3.

(10) No formation of ethylene glycol mono- or diacetates is observed. These products might arise from oxidation of the Pd–C bond in the $[X_2PdCH_2CH_2OAc]^-$ intermediate by cobalt–nitro complexes in analogy to the behavior of $LiNO_3$; see: Tarura, M.; Yasui, T. *Kogyo Kagaku Zasshi* **1969**, *72*, 575, 578, 581, 585.

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Received June 9, 1980

A Direct NMR Method for the Determination of Correlation Times in Enzyme Complexes Involving Monovalent Cations and Paramagnetic Centers

Sir:

A persistent problem associated with using NMR methods to determine internuclear distances between paramagnetic centers and ligands bound to a macromolecule is the determination of the correlation time, τ_c, for the electron–nuclear interaction.[1] Practice has shown that the dipolar electron–nuclear interaction dominates the relaxation processes for several paramagnetic species and that relaxation can be described by the Solomon–Bloembergen equation.[2] The simplified form of this equation applicable to longitudinal relaxation data obtained with paramagnetic centers and $\tau_c \geq 10^{-10}$ s is given by eq 1, where r is the electron–nuclear

$$r = C\left[T_{1M}\frac{3\tau_c}{1+\omega_I^2\tau_c^2}\right]^{1/6} \quad (1)$$

distance, C is a collection of constants whose value depends on the spin of the paramagnetic center and the gyromagnetic ratio of the nucleus, T_{1M} is the spin–lattice relaxation time attributable to the influence of the paramagnetic species, and ω_I is the nuclear Larmor precession frequency. This treatment also assumes fast exchange conditions and therefore that $1/T_{1p} = 1/T_{1M}$, where $1/T_{1p}$ is the observed relaxation rate corrected for diamagnetic effects.

Several methods have been described to estimate a value of τ_c for use in calculating distances: (1) measurement of T_1 and T_2 values for the nuclei, (2) a frequency dependence of T_1 of the nuclei, (3) a frequency dependence of the relaxation rates of solvent water in the system, and (4) measurement of the line width of the EPR spectrum of the paramagnetic species. Of these, the measurement of the frequency dependence of T_1 of the nuclei under observation gives the best results but of course requires that data be obtained by using two spectrometers or a single spectrometer with a variable field electromagnet. Also, for some ions, e.g., Mn^{2+} and Cu^{2+}, the relevant correlation time for the dipolar relaxation is the electron spin relaxation time which itself can be magnetic field dependent, further complicating the data analysis.

With these considerations in mind, we pursued NMR studies of several alkali metal ions in an attempt to measure distances between the monovalent–divalent cation sites of pyruvate kinase. Recently, two laboratories have reported the internuclear distance between Mn^{2+} and $^7Li^+$ bound to pyruvate kinase from rabbit muscle.[3] Ash et al.[3b] obtained a distance of 4.7 Å while Hutton et al.[3a] obtained a distance of 5.8 Å. The major reason for the difference in the measured distance is in the choice of the correlation time for the dipolar interaction. On the basis of water proton relaxation rate studies, Ash et al.[3b] and Hutton et al.[3a] used a τ_c value of 1.7 and 9.4 ns, respectively.

It occurred to us that an unambiguous determination of the correlation time could be made by performing identical experiments with $^6Li^+$ and $^7Li^+$ at the same magnetic field strength, thus obviating many of the problems discussed above.[4] From the ratio of the observed T_{1p} (T_{1M}) values for $^6Li^+$ and $^7Li^+$, a unique value of τ_c is obtained because in eq 1 C and ω_I are known values for the two isotopes of lithium, and the distance r must be the same

(1) Dwek, R. A. "Nuclear Magnetic Resonance in Biochemistry", Clarendon Press: Oxford, 1973; Chapter 10.

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

(3) (a) Hutton, W. C.; Stephens, E. M.; Grisham, C. M. *Arch. Biochem. Biophys.* **1977**, *184*, 166. (b) Ash, D. E.; Kayne, F. J.; Reed, G. H. *Ibid.* **1978**, *190*, 571.

(4) Reuben, J. *J. Chem. Phys.* **1975**, *63*, 5063. Reuben measured ^1H and ^2H relaxation rates at one magnetic field strength in order to determine if $\tau_M > T_{1M}$ in aqueous solutions containing Gd^{3+}. In principle, our approach is analogous to this, but we apply the multiple isotope method to determine the correlation time for the dipolar relaxation induced by a paramagnetic species on monovalent cations in macromolecular complexes. The method we describe is valid for any two isotopes that have the same chemical properties in the system under analysis, e.g., both are cations or both are organic derivatives with ^{14}N or ^{15}N isotopes, and so forth. This method demands that $\tau_M > T_{1M}$.

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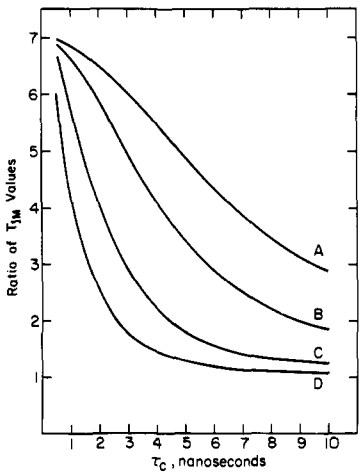

Figure 1. Determination of the correlation time (τ_c) for the dipolar interaction of enzyme–Mn^{2+} from the ratio of T_{1M} for $^6Li^+$ and $^7Li^+$ at various magnetic field strengths; (A) 14 kG, (B) 23 kG, (C) 47 kG, (D) 85 kG.

for both ions. It is assumed that τ_c is the same for both ions. Shown in Figure 1 is a theoretical plot of the ratio of T_{1M} values for $^6Li^+$ and $^7Li^+$ at various magnetic field strengths vs. a range of correlation times commonly found in enzyme–Mn^{2+} complexes. Examination of the figure shows that this method is very sensitive for the determination of τ_c values of $<10^{-8}$ s since in this range the T_{1M} values for $^6Li^+$ and $^7Li^+$ are quite different. Similar plots can also be constructed for the two isotopes of NH_4^+ (^{15}N and ^{14}N) and Rb^+ (^{87}Rb and ^{85}Rb).

We have measured the T_{1p} values for both $^6Li^+$ and $^7Li^+$ in the pyruvate kinase–Mn^{2+}–phospho(enol)pyruvate–Li^+ complex at a magnetic field strength of 47 kG (29.45 MHz for ^6Li, 77.77 MHz for ^7Li) by using a multinuclear Brüker WP-200 NMR spectrometer.[5] The T_{1p} values were calculated by using eq 2,

$$f(1/T_{1p}) = 1/T_{1(Mn)} - 1/T_{1(Mg)} \quad (2)$$

where $T_{1(Mn)}$ and $T_{1(Mg)}$ are observed spin–lattice relaxation times[6] in the presence of either Mn^{2+} or Mg^{2+} bound to the enzyme, and f is the mole fraction of enzyme-bound Li^+. The observed $1/T_{1p}$ values at 30 °C for the enzyme complexes with $^7Li^+$ and $^6Li^+$ are $(3.2 \pm 0.3) \times 10^3$ and $(1.3 \pm 0.1) \times 10^3$ s^{-1}, respectively.[7] The ratio of these T_{1p} values is 2.4; thus, a τ_c of $(3.7 \pm 0.6) \times 10^{-9}$ s is calculated from Figure 1 (assuming fast chemical exchange).[8] The distance between enzyme-bound Mn^{2+} and Li^+ is 5.7 ± 0.2 Å, using eq 1 and the values obtained for T_{1M} and τ_c. This is in agreement with the results of Hutton et al.[3a]

This distance of 5.7 Å is longer than the previously determined distance of 4.9 Å between Mn^{2+} and $^{205}Tl^+$ with pyruvate kinase.[9] Since Li^+ activates the enzyme only 3% as well as Tl^+,[10] this suggests that a substantial difference in the orientation between the divalent and monovalent cations at the active site of pyruvate kinase could be responsible for the large difference in maximal activities. Different enzyme conformations stabilized by Tl^+ and Li^+ are indicated by the dissimilar Mn^{2+}–Li^+ and Mn^{2+}–Tl^+ distances. We are now in the process of extending these studies to several other monovalent cations that activate pyruvate kinase.

The new method that we report in this communication for the accurate measurement of τ_c should be applicable to quite a number of enzymes that are activated by monovalent cations. Also, since the measurements are made at one magnetic field strength,[11] the field dependence of the correlation time is no longer a potential source of error.

Acknowledgment. This research was supported in part by grants from the National Science Foundation (for instrumentation and research, PCM-7807845). J.J.V. is an Established Investigator of the American Heart Association. F.M.R. is a National Research Service Awardee (AM-05966).

(5) Spin–lattice relaxation measurements were performed on 1.25-mL samples in 10-mm NMR tubes containing 50 mM tris(hydroxymethyl)-aminomethane, pH 7.5, 20% D_2O, 100 mM ^7LiCl or ^6LiCl (obtained from Oak Ridge National Laboratory), 1.0 mM phospho(enol)pyruvate, 40 μM (in sites) pyruvate kinase (obtained from Sigma Chemical Co.), and either 200 μM $MnCl_2$ or 2.5 mM $MgCl_2$. When $MgCl_2$ was used, 1.0 mM EDTA was also added to remove any bound paramagnetic impurities that may have been introduced with the enzyme.

(6) The spin–lattice relaxation times were determined with a 180°–τ–90° pulse sequence. The relaxation times for $^6Li^+$ were 93 and 2.0 s in the enzyme complexes with Mg^{2+} and Mn^{2+}, respectively. For $^7Li^+$, the relaxation times were 10.8 and 0.79 s for these same enzyme complexes. The mole fraction of enzyme-bound Li^+ was calculated by using a dissociation constant of 11 mM.[3a]

(7) The experimental $1/T_{1p}$ values have not been corrected for the relative amounts of the two interconvertible forms of the enzyme–Mn^{2+}–Li^+–PEP complex[3b] because of the large uncertainty in the actual size of the correction. If the maximum possible correction is made, the $1/T_{1p}$ values for Li^+ increase by only 19%, and the distance decreases by 0.2 Å.

(8) The large T_{1p}/T_{2p} ratios found in Ash et al.[3b] indicate that the fast exchange conditions prevail in this system.

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

(10) Kayne, F. J. *Enzymes, 3rd Ed.* **1970–1976**, *8*, 353–382.

(11) Multinuclear spectrometers are becoming common place in several laboratories, and, thus, this approach should be widely applicable.

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Received May 5, 1980

Theoretical Models for Transition-State Structure and Catalysis in Carbonyl Addition

Sir:

The formation of methanediol from water and formaldehyde (eq 1) is suggested by ab initio self-consistent field molecular orbital calculations at the 4-31G level[1] to be a quite difficult

$$H_2O + CH_2O \rightarrow CH_2(OH)_2 \quad (1)$$

process, passing through a transition state[2,3] for simultaneous proton transfer and carbon–oxygen bond formation. A significant feature of the transition-state structure (Figure 1) is the presence of an essentially planar four-membered ring formed between the O–H bond of the attacking water molecule and the C–O bond of formaldehyde. Furthermore, the water is oriented in such a fashion as to enable one of its lone pairs to align approximately along the newly forming O–C bond. A rather large activation energy of 44.1 kcal mol^{-1} exists for water–formaldehyde addition, and the reaction is exothermic by 16.8 kcal mol^{-1}.

If proton transfer is disallowed by imposing a C_s symmetry constraint on the approach of water to formaldehyde, carbon–oxygen bond formation would yield a zwitterionic adduct. However, as shown by curve (d) of Figure 2, the interaction of water with formaldehyde as a function of carbon–oxygen distance[4] is wholly repulsive along this path. This zwitterionic structure and its rotamers, which were also examined, are therefore unbound states, and reaction through the transition state of Figure 1 is an example of *enforced concertedness*[5] of proton transfer and heavy-atom bond formation. Because the zwitterionic intermediate does not exist, generation of methanediol by C–O bond formation and proton transfer in two discrete steps cannot occur. Thus, the

(1) Ditchfield, R.; Hehre, W. J.; Pople, J. A. *J. Chem. Phys.* **1971**, *54*, 724–728.

(2) The calculated structure satisfies the requirements for a transition state, as discussed in a number of works. See, for example: Maggiora, G. M.; Christoffersen, R. E. "Transition States in Biochemical Processes"; Gandour, R. D., Schowen, R. L., Eds.; Plenum Press: New York, 1978; Chapter 3.

(3) The calculations were carried out with a modified version of HONDO/G on the NRCC's VAX 11/780 computer, using a gradient-search procedure.

(4) The carbon–oxygen distance is a chemically reasonable coordinate with which to follow reaction progress, although it is not a true "reaction coordinate"; see, for example: Miller, W. H.; Handy, N. C.; Adams, J. E. *J. Chem. Phys.* **1980**, *72*, 99–112. The subject will be discussed in greater detail in a forthcoming paper.[6]

(5) Jencks, W. P. *Acc. Chem. Res.* **1976**, *9*, 425; *Ibid.* **1980**, *13*, 161.

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