In both cases, as soon as the energy-transfer process is complete, the acceptor is subject to fluorescence depolarization due to movements during the lifetime of its excited singlet state.[3] If we are to reason that solvents of low viscosity allow for a higher probability of preferred dipole–dipole orientations, we must also take into account this "classical" viscosity effect operating in the opposite direction. Hence, the value of P_{ET} might be expected to reach a maximum at some intermediate solvent viscosity. This is observed in the case of 17 α-naphthylyohimbol. Reserpine shows a general increase in P_{ET} in going toward less viscous solvents, but the results are somewhat erratic, perhaps due to the presence of favored conformations for different solvents, and the molecule's greater over-all flexibility. Nevertheless, we conclude that the results for both molecules indicate an enhancement of efficiency of energy transfer due to the attainment of favorable electronic transition dipole–dipole orientations of the donor and acceptor during the energy-transfer process.

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Nuclear Magnetic Resonance Study of Calcium-43

Sir:

Nuclear magnetic resonance methods have become an important means of investigating a variety of interactions that are biochemically significant. While proton resonance measurements offer the greatest sensitivity, nuclei such as the halogens offer the advantages of selectively amplifying certain interactions with sites associated with large molecules such as enzymes or proteins.[1] With the exception of sodium-23 measurements[2] little progress has been made in utilizing magnetic resonance of alkaline or alkaline earth cations for probing the environment of these ions in the presence of macromolecules. Because calcium ion has been critically implicated in a variety of life processes, a study of the calcium-43 isotope was undertaken to determine the suitability of nmr as a method of directly studying the calcium environment in systems of biological interest. This note reports measurements of calcium-43 relaxation times in aqueous adenosine triphosphate solutions which indicate that the calcium-43 relaxation times are such that nmr should be a powerful approach for the study of biologically significant calcium complexes.

Calcium-43 was purchased as the carbonate 31.68% enriched from the Union Carbide Corp. at Oak Ridge National Laboratories. Adenosine triphosphate was obtained as the disodium salt from Calbiochem; all other chemicals used were Baker and Adamson analyzed reagents. The calcium concentration in the solutions studied was 0.91 *M*. The ATP solutions were made by successive additions of the solid to the initial calcium solution; the pH was then adjusted by addition of sodium hydroxide. In adjusting the pH the molar concentration of the calcium decreases; however, the ATP concentration decreases by the same factor so that the ratio ATP/Ca remains the same.

The nmr spectrometer employed a Varian V-4210A variable-frequency radiofrequency unit crystal locked at 4.00 MHz, a Varian 12-in. magnet, flux stabilizer, slow sweep unit, and homogeneity control unit. A Princeton Applied Research Model JB-4 lock-in amplifier was used in conjunction with a Dynakit audioamplifier and a Hewlett-Packard Model 201CR audio oscillator to stabilize the base line. Spectra were recorded using a Sanborn Model 7700 recorder in order to provide a rapid response time. Relaxation times were measured using the method described by Sykes[3] and the errors shown are mean deviations from the mean of three or more separate measurements.

In strongly acidic solutions the calcium-43 T_2 is 1.2 ± 0.1 sec. A measurement of the calcium-43 relaxation time is shown in Figure 1 where no precautions were taken to exclude carbonate and T_2 is 0.85 ± 0.1 sec. The pH was adjusted to 6.4 and the relaxation time measured as a function of adenosine triphosphate concentration; the data are shown in Figure 2.

Calcium-43 has a nuclear spin of $^7/_2$ which implies that the nucleus may possess a quadrupole moment. With T_2 as long as 1 sec it is possible that there are other than quadrupolar contributions to T_2. In solid CaF_2, however, the quadrupole interaction is the dominant relaxation mechanism,[4] and for environments other than solvated calcium ion the quadrupolar mechanism should dominate. For a nucleus that is quadrupole relaxed, the transverse relaxation time in sec^{-1} is given by eq 1, where q is the

$$\frac{1}{T_2} = \frac{2\pi^2}{49}(e^2qQ)^2\tau_c \qquad (1)$$

electric field gradient at the nucleus with quadrupole moment Q, τ_c is the correlation time for the reorientation of the field gradient with respect to the applied field, and the asymmetry parameter has been neglected.[5]

The linear dependence of the relaxation time on the ATP concentration is explained by the exchange of the calcium free in solution with the calcium bound to the phosphate portion of the ATP molecule. The exchange rate as measured by temperature-jump techniques is greater than 2.5×10^5 sec^{-1}, and this rate is sufficiently

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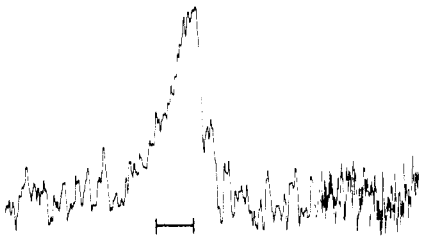

Figure 1. A typical measurement of T_2 for calcium-43 in aqueous solution; $T_2 = 0.85$ sec. Mark equals 1 sec.

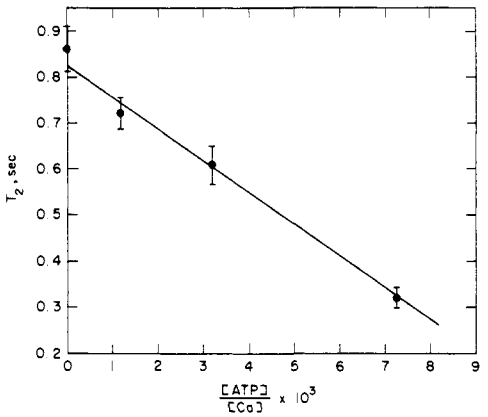

Figure 2. The transverse relaxation of calcium-43 as a function of ATP concentration at pH 6.4.

rapid for the rapid exchange approximation to obtain.[6] In this fast exchange limit the observed relaxation time is a weighted average of the relaxation times that characterize each site in the spectrum

$$\frac{1}{T_2} = \sum_{\text{sites}} P_i \left(\frac{1}{T_2}\right)_i \qquad (2)$$

where P_i is the probability that a calcium-43 ion is found at site i with a relaxation rate $(1/T_2)_i$.[7] Equation 2 may be simplified to the sum of two terms to permit estimation of an average $1/T_2$ for the ATP site.[8] At this pH $(1/T_2)_{atp}$ is 262 sec^{-1}. Since the exchange rate of calcium with ATP is about three orders of magnitude more rapid than $(1/T_2)_{atp}$, the fast-exchange approximation will obtain even if there is an increase in the product $(e^2qQ)^2\tau_c$ by about three orders of magnitude. Assuming that values of the field gradient do not vary drastically between one type of phosphate and another, the fast exchange approximation may be applied even if τ_c increases by three orders of magnitude, as it might if ATP were to bind to an enzyme for example. This suggests that enzyme- or protein-bound calcium should be observable at physiologically reasonable concentrations of protein because the exchange serves as a chemical amplifier in the same way that chemical exchange of chloride amplifies the chloride ion interaction with bovine plasma albumin.[9] As with other rapid exchange systems such as the halogen ion probe technique,[9,10] appropriate control experiments may serve to isolate the bound calcium contribution to the total relaxation time. This contribution then reflects the accessibility of the metal to the exchange site, the electric environment of the metal site, and the mobility of the site.

The binding of calcium to ATP is certainly a very well-known phenomenon; however, this experiment has shown that calcium nuclear magnetic resonance is potentially a powerful probe for investigating the calcium environment in systems where the calcium interactions may be important. Furthermore the exchange rate of calcium with potential binding sites is likely to be rapid enough so that the observation and characterization of bound calcium should be possible when the binding site is protein, enzyme, or even tissue such as muscle or nerve fiber.

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Diastereomeric Solute–Solute Interactions of Enantiomers in Achiral Solvents. Nonequivalence of the Nuclear Magnetic Resonance Spectra of Racemic and Optically Active Dihydroquinine

Sir:

It is generally accepted that the nmr spectra of a racemate and of either one of its enantiomers are identical when measured under the same conditions in an achiral solvent.[1] For instance, it is now a matter of routine to establish the structural identity of an optically active natural product and its corresponding synthetic racemate by comparing their nmr spectra. We report that our data refute this method as a principle. The nmr spectra of optically active dihydroquinine and of racemic dihydroquinine[2] are significantly different when taken at the same

(1) In chiral solvents[1a] or upon addition of chiral additives in achiral solvents,[1b] two enantiomers reside in diastereomeric environments and, hence, can in principle be distinguished by their nmr spectra. Recently these phenomena were applied to the determination of optical purity[1c] and to the assignment of absolute configuration by nmr spectroscopy.[1d] (a) K. Mislow and M. Raban in "Topics of Setreochemistry," Vol. 1, N. L. Allinger and E. L. Eliel, Ed., Interscience Publishers, New York, N. Y., 1967, p 22 ff, and references therein; (b) J. C. Jochims, G. Taigel, and A. Seeliger, *Tetrahedron Lett.*, 1901 (1967); F. A. L. Anet, L. M. Sweeting, T. A. Whitney, and D. J. Cram, *ibid.*, 2617 (1968); W. H. Pirkle and S. D. Beare, *J. Amer. Chem. Soc.*, **90**, 120 (1968); (c) M. Raban and K. Mislow in "Topics in Stereochemistry," Vol. 2, N. L. Allinger and E. L. Eliel, Ed., Interscience Publishers, New York, N. Y., 1967, p 216, and references therein; (d) W. H. Pirkle and S. D. Beare, *J. Amer. Chem. Soc.*, **89**, 5485 (1967).