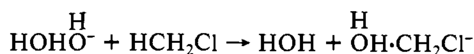
because it would be weakly held, the chlorine withdrawing the negative charge from the site of the proton transfer.



$$\mathrm{HOH\overset{}{O}^- + HCH_2Cl \rightarrow HOH + \overset{H}{O}H{\cdot}CH_2Cl^-}$$

In conclusion, the reactivity of a Lewis base, in its competing roles of Brønsted base and nucleophile, has been explored as a function of translational energy. The rule at thermal energies that proton transfer prevails where it is spontaneous[3] is here extended to suprathermal energies, where proton transfer *within the intermediate* must be spontaneous for the reactants. Adding a single solvate molecule is again shown to change a reaction mechanism[22] —here, apparently, by perturbing differentially the energies of the participating species.

Acknowledgment. We appreciate the critical comments of Professor Grabowski. M.H. acknowledges support from NIH (BRSG S07RR07044).

Registry No. CH_3Cl, 74-87-3; OH^-, 14280-30-9.

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Mechanism of Adenylate Kinase. 1. Use of ^{17}O NMR To Study the Binding Properties of Substrates[1]

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Despite the ubiquity of oxygen-containing functional groups (e.g., phosphoryl, carboxyl, and hydroxyl groups) in biological systems, ^{17}O NMR has not been used to study the binding and motional properties of enzyme–substrate complexes. On the other hand, binding of small ligands to proteins has been investigated by the NMR properties of other quadrupolar nuclei such as ^{43}Ca,[2] ^{79}Br, ^{81}Br,[3] ^{35}Cl,[4] ^{2}H,[5] etc.

Adenylate kinase (AK) provides a good system to test the applicability of ^{17}O NMR in enzyme–substrate interactions. The enzyme is small ($M_r \approx 21\,000$), yet consists of two distinct sites: the MgATP site binds ADP, ATP, MgADP, and MgATP, whereas the AMP site binds AMP and ADP.[6] The dissociation constants are in the order of 10^{-4}–10^{-5} M.[6b,c]

The ^{17}O line width (ΔO) in the extreme narrowing limit ($\omega^2\tau_c^2 << 1$) can be expressed by[7]

$$\Delta O = \frac{1}{\pi T_2} = \frac{1}{\pi T_1} = \frac{12\pi}{125}\left(1 + \frac{\eta^2}{3}\right)\left(\frac{e^2qQ}{h}\right)^2 \tau_c \quad (1)$$

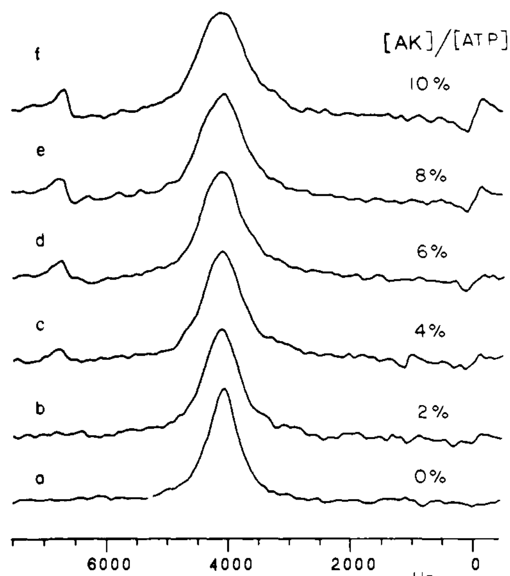

Figure 1. ^{17}O NMR spectra (40.7 MHz) of [β-$^{17}O_2$]ATP in the presence of various concentrations of AK. Sample conditions: (a) 7.0 μmol of [β-$^{17}O_2$]ATP in 0.20 mL of 100 mM Hepes buffer, pH 7.9, containing 14 μmol of EDTA; (b–f) addition of 2.94 mg of AK (0.14 μmol) in 0.132 mL of 100 mM Hepes buffer (pH 7.9). ^{17}O-depleted water (10% ^{17}O relative to natural abundance) was used in all cases. Spectral parameters: spectral width 25 000 Hz, acquistion time 41 ms, acquisition delay 20 ms, receiver gate 40 μs. The T_1 inversion-recovery program was used to suppress the solvent signal (180° = 51 μs, 90° = 25.5, μs, recovery time 4.5 ms). Temperature was 20 °C, line broadening 100 Hz, no. of transients 12 000–25 000. The instrument and probe have been described elsewhere.[19]

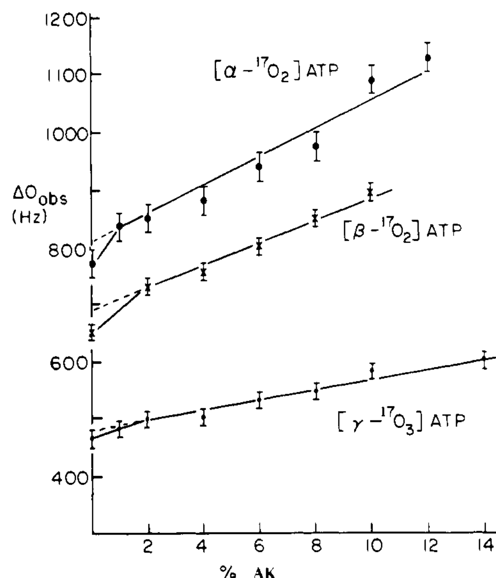

Figure 2. Dependence of ^{17}O NMR line widths, ΔO, on the ratio P = [AK]/[nucleotide]. The conditions and spectral parameters are similar to Figure 1. The ^{17}O-labeled nucleotides were available from our previous work,[19] with all ^{17}O label at nonbridging positions of the phosphate groups. The ΔO values have not been corrected for exponential multiplication (100 Hz) and field inhomogeneity (20 Hz).

where ω is the angular frequency of ^{17}O, τ_c is the rotational correlation time, and η and e^2qQ/h are the asymmetry parameter and the quadrupolar coupling constant, respectively, of the ^{17}O nucleus. When a small percentage (P) of an ^{17}O-labeled nucleotide is bound to an enzyme, the observed line width is given by[8-10]

(1) This work was supported by research Grant GM 29041 from NIH. M.-D.T. is an Alfred P. Sloan Fellow, 1983–1985. Abbreviations: ADP, adenosine 5′-diphosphate; AK, adenylate kinase; AMP, adenosine 5′-monophosphate; ATP, adenosine 5′-triphosphate; EDTA, ethylenediaminetetraacetate; GTP, guanosine 5′-triphosphate; Hepes, N-(2-hydroxyethyl)piperazine-N-2-ethanesulfonic acid; PPP, triphosphate; T_1, spin–lattice relaxation time; T_2, spin–spin relaxation time.

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$$\Delta O = \frac{1}{\pi T_{2,\mathrm{obsd}}} = \frac{1}{\pi}\left(\frac{1}{T_{2f}} + \frac{P}{T_{2b} + \tau}\right) \quad (2)$$

Depending on the relative values of the lifetime of the bound nucleotide (τ) and the transverse relaxation times of free and bound nucleotides (T_{2f} and T_{2b}, respectively), plots of ΔO vs. P may allow calculation of T_{2b} or τ.

Figure 1 shows the ^{17}O NMR signal of [β-$^{17}O_2$]ATP titrated with small percentages of AK purified from porcine muscle.[11] The successive line broadening is consistent with eq 2, whereas the Lorentzian line shapes suggest that the broadening is not due to chemical exchange and that the condition $\omega^2\tau_c^2 << 1$ is met.[8] Figure 2 shows the plots of ΔO vs. P for [γ-$^{17}O_3$]ATP, [β-$^{17}O_2$]ATP, and [α-$^{17}O_2$]ATP. In all cases the ΔO increases upon the first addition of AK due to an increased viscosity. With further addition of the enzyme solution, the change in viscosity is less significant,[12] yet the signals of all three samples of ATP (without Mg^{2+}) show an approximately linear increase in ΔO. The result of [γ-$^{17}O_3$]ATP has been confirmed by titrating an enzyme solution with a negligible volume of ATP solutions, which rules out the viscosity change as the predominant factor in causing the line broadening. At the end of each experiment, neither hydrolysis of ATP nor deactivation of AK was detectable.

The slopes of the plots of the ΔO of ATP represent $1/\pi(T_{2b} + \tau)$. The fact that the slopes are *different* for α-, β-, and γ-^{17}O of ATP suggests that T_{2b} instead of τ is the predominant factor in causing the line broadening; i.e., $\tau << T_{2b}$. The slopes are 2500, 1950, and 900 Hz for α-, β-, and γ-^{17}O of ATP, respectively. The ΔO_f, obtained from the signals of free ATP after correcting for 100 Hz of line broadening and 20 Hz of inhomogeneity, is 690, 570, and 355 Hz for α-, β-, and γ-^{17}O of ATP, respectively. Although the slopes may be considered as ΔO_b, it is more accurate to obtain ΔO_b by extrapolating the linear curves in Figure 2 to 100% AK, particularly when ΔO_f is not negligible compared to ΔO_b. The ΔO_b values thus obtained (after correction) are 3200, 2520, and 1250 Hz for α-, β- and γ-^{17}O of ATP, respectively.

If it is assumed that the differences between ΔO_f and ΔO_b are mainly due to changes in τ_c, the results suggest the τ_c of the ^{17}O of ATP increases by only a factor of 3–5 upon binding to AK. The values could be as high as 10–20 if there is a decrease in e^2qQ/h due to H-bonding or other reasons.[13,14] Since the increase in τ_c should be in the order of 10^2 if binding is rigid,[15] the triphosphate moiety of ATP most likely has appreciable internal rotational freedom in the AK–ATP binary complex. This is consistent with the previous finding that the ^{31}P NMR properties of ATP without (Mg^{2+}) change only slightly upon binding with AK[6a] and suggests that the phosphate moiety of bound ATP may interact only weakly with the enzyme in the absence of Mg^{2+}.[16]

A major concern in the use of ^{17}O NMR in macromolecular systems is that the simple eq 1 is no longer valid when $\omega\tau_c \geq 1$.[8,10b,17] This seems to be less of a problem for small enzymes such as AK. The upper limit of $\omega\tau_c$ is ca. 1 at 40 MHz if the substrate assumes the same τ_c as the enzyme molecule (15–20 ns).[18] In many cases,[3] such as the one described in this paper, the bound substrate can assume a certain degree of rotational freedom (relative to the enzyme) and justifies the use of eq 1. The requirement that is hard to meet seems to be the "fast exchange limit" ($\tau << T_{2b}$). Although it is the case for ATP, we have found that the ΔO of [γ-$^{17}O_3$]MgATP and [$^{17}O_3$]AMP are less sensitive to additions of AK, which suggests that the latter two cases are probably in the "slow exchange limit" due to a long τ, a short T_{2b}, a large chemical shift change,[8,9] or a combination of these.

(9) As pointed out in ref 8, another requirement for eq 2 to be valid is that the difference in chemical shift between the free and the bound state ($\Delta\delta$) is less than $1/T_{2b}$. The ^{17}O chemical shifts of nucleotides rarely change by >50 ppm.[10]

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(12) A small volume of ATP solution was used in order to minimze changes in viscosity. According to the sample conditions described in Figure 1, the concentrations of AK at 2% and 10% (relative to ATP) were 0.42 and 0.82 mM, respectively. Thus, the viscosity change should show a "quantum increase" at 2% AK and become negligible later.

(13) The nuclear quadrupolar coupling constant of ^{17}O could decrease for two possible reasons. The first is hydrogen bonding between the phosphate moiety of ATP and the active site residues. A model for the H-bonding effect is that the e^2qQ/h value for $H_2{}^{17}O$ is 10.17 MHz in the gas phase and 6.525 MHz in ice.[14a] The effect of H-bonding on the quadrupolar coupling constant of ^{17}O has been studied in detail.[14b] The second possible reason is that the charges on the phosphate of ATP are localized (to O=P—O⁻) due to ionic interactions with positive residues at the active site and that only the signal due to P=^{17}O is being observed (the other, P—^{17}O⁻, may not be observable due to much shorter relaxation times). A model for this is $(PhO)_3PO$, where the e^2qQ/h values are 3.825 MHz for P=^{17}O and 9.176 MHz for P—^{17}O—Ph (average of three values[14c]). The e^2qQ/h values for KH_2PO_4 are 4.85–5.96 MHz.[14d]

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(16) The dissociation constants of the AK complexes with ATP, GTP, and PPP (without Mg^{2+}) are 35, 1200, and 240 μM, respectively.[6c] This suggests that the adenine moiety is important in binding. The triphosphate also binds to AK, but it is not known whether its binding is limited to the ATP site or it crosses both AMP and ATP sites.

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Axial and Equatorial 4-*tert*-Butyladamantan-2-ones. Synthesis, Circular Dichroism, and Mass Spectra[1]

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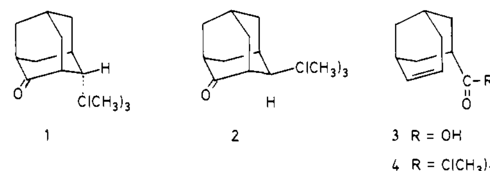
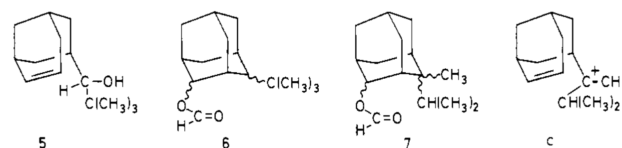
Our interest in unusual (anti-octant[2]) effects in the circular dichroism (CD) spectra of saturated alkyl ketones led us to reexamine[3] the previously reported[4] solvent-dependent anti-octant effects seen with 4(a)-methyl-adamantan-2-one and to extend our investigations to the corresponding *tert*-butyladamantan-2-one. Both 4(a)- and 4(e)-*tert*-butyladamantan-2-one (**1** and **2**) were





unknown at the time of our work, and they are of special interest because (1) no chair cyclohexanones have been prepared with a β-axial *tert*-butyl group and (2) the *tert*-butyl substituent exhibits the same general symmetry as the methyl group but is more

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