We have found that high molecular weight polysilanes are rapidly degraded in the presence of ultrasound (cf. Table II). A similar effect has previously been observed for polystyrene, poly(methylmethacrylate), dextran, and other polymers.[11] Selective degradation is of a mechanical nature caused by friction forces between macromolecules and solvent molecules during the cavitation process. Larger molecules are more resistant to flow, have larger shear forces, and rupture more frequently than shorter macromolecules. Beyond certain molecular weight, shear forces are smaller than bond strengths, and polymers cannot degrade. This selective degradation not only reduces molecular weights to a certain value but also descreases polydispersity.

Thus, although low polydispersities can be explained by the selective degradation of polysilanes, the absence of low molecular weight polymers has the origin in the suppression of side reactions and probably in the promoting of the polymerization with anionic intermediates.

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**Registry No.** $PhMeSiCl_2$(homopolymer), 31324-77-3; $PhMeSiCl_2$-(SRU), 76188-55-1; $(n\text{-Hex})_2SiCl_2$(homopolymer), 97036-67-4; $(n\text{-Hex})_2SiCl_2$ (SRU), 94904-85-5; $(PhMeSiCl_2)((n\text{-Hex})_2SiCl_2)$(copolymer), 113925-33-0; $(PhMeSiCl_2)(Ph_2SnCl_2)$(copolymer), 113925-34-1.

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## Mechanism of Adenylate Kinase. 3. Use of Deuterium NMR To Show Lack of Correlation between Local Substrate Dynamics and Local Binding Energy[1]

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The fact that enzymes utilize binding energy to bring about entropically unfavorable events and thus facilitate catalysis[2] suggests that enzymes are fundamentally involved in the control of substrate motion. We have examined the local motions of AMPPCP bound to adenylate kinase (AK) in order to examine the relationships between local rigidity of a bound substrate, binding energy, and catalysis by AK.

Chicken muscle AK was titrated with AMPPCP[3] deuteriated upon the phosphonate chain and upon the adenine ring[4] and followed by measuring the deuterium NMR line width ($\Delta\nu_{1/2}$) of the single peak which results from the average of the bound and free AMPPCP. Plots of $\Delta\nu_{1/2}$ versus $[AMPPCP]_{bound}/[AMPPCP]_{total}$ were linear, as shown in Figure 1. The line shapes of the observed peaks were usually Lorentzian, and limited $T_1$ inversion recovery data taken were always monoexponential. Upon increasing temperature from 5 °C to 35 °C, the line widths decreased with a magnitude approximately proportional to the decreasing solution viscosity. Thus, our data meet "fast-exchange" criteria, and the line widths of the fully bound species can be determined from linear extrapolations to fraction bound = 1.[5] Indeed very little extrapolation is required for two of the curves. The line widths obtained are listed in Table I. The effective rotational correlation times ($\tau_c$) for isotropic motions were then calculated from the well-known relationship between line width ($1/\pi T_2$) and $\tau_c$.[6-8] The contributions of two-bond $^2H-^{14}N$ and $^2H-^{31}P$ scalar and dipolar couplings to the observed $\Delta\nu_{1/2}$ were insignificant. The validity of the $\tau_c$ data determined from such analysis was further supported by the fact that the same $\tau_c$ values (within experimental errors) were obtained from $T_1$ experiments

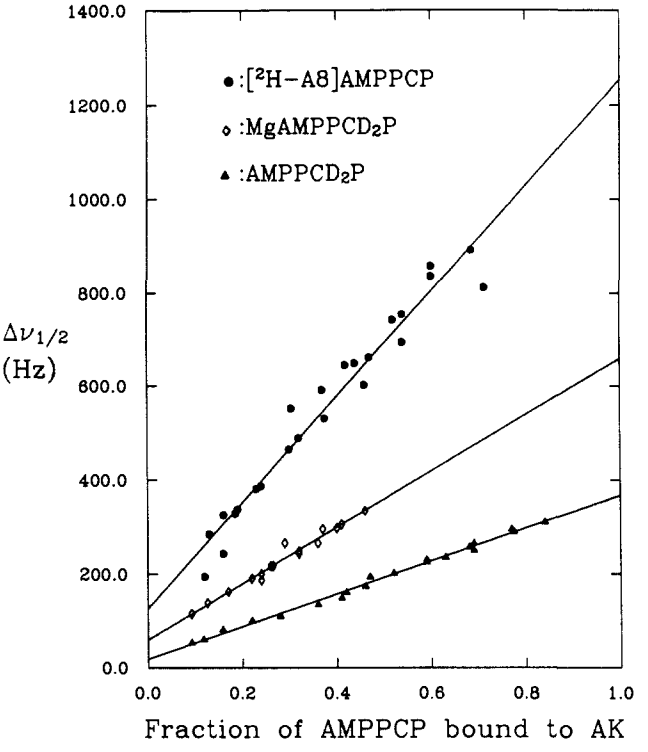


**Figure 1.** $^2H$ NMR (46.1 MHz) line widths ($\Delta\nu_{1/2}$ in Hz) of $^2H$-labeled AMPPCP and MgAMPPCP ($[Mg^{2+}]/[AMPPCP] = 4$) as a function of fractions bound to AK, obtained by titrating 1-2 mM AK with the nucleotides. Sample conditions: pH 7.0 in $^2H$-depleted $H_2O$ with 45 mM Hepes-$K^+$ or imidazole-HCl, 117 mM KCl, 1-8 mM dithiothreitol, and 0.1 mM EDTA in a 10-mm NMR tube (starting volume of 1.75 ml) at 10 °C. Spectral conditions: digital resolution 1 Hz/point (narrow signals) to 15 Hz/point (very broad signals), 90 °C pulse width 12 μs. The reported $\Delta\nu_{1/2}$ have been corrected for line broadening (1-10 Hz). The fraction of AMPPCP bound to AK was calculated by using $K_d$ values of 210 μM for AMPPCP and 190 μM for MgAMPPCP determined in our lab and elsewhere.[19,25]

(1) Abbreviations: AMPPCP, adenylyl (β,γ-methylene)diphosphonate; AK, adenylate kinase; ATP, adenosine 5'-triphosphate; AMP, adenosine 5'-monophosphate; EDTA, ethylenediamine tetraacetate; Hepes, *N*-(2-hydroxy ethyl)piperazine-*N*-2-ethanesulfonic acid; PPPi, triphosphate; $T_1$, spin-lattice relaxation time; $T_2$, spin-spin relaxation time.

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(3) It is assumed that AMPPCP interacts with AK in a motionally and energetically similar manner as ATP, since the observed binding energies (based on $K_d$) for ATP and AMPPCP are similar (-5.2 and -5.1 kcal/mol, respectively).

(4) [8-$^2H$]AMPPCP was prepared by heating AMPPCP in $D_2O$ at $pH_{obsd}$ = 10 for 3-6 h at 95 °C. $AMPPCD_2P$ was synthesized by modified literature procedures.[20,21]

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(7) We assume effectively isotropic motion (on an NMR time scale) since $\tau_c \ll$ [quadrupolar coupling constant]$^{-1}$. We also assume purely quadrupolar relaxation because of the relatively high quadrupolar coupling constants exhibited by deuteriated hydrocarbons due to aspherical electronic symmetry and because there are no reasons to expect a competing mechanism. For our calculations we utilized an asymmetry parameter of zero which is quite reasonable due to the axial symmetry of the electric field gradient of the deuterium nuclei in our compounds. Finally, we assume quadrupolar coupling constants of 178 MHz for [8-$^2H$]AMPPCP and 168 MHz for both $AMPPCD_2P$ and $MgAMPPCD_2P$. These values were chosen through chemical analogy with [8-$^2H$]AMP[22] and deuteriated malonic acid.[23] This is reasonable since $^2H$ quadrupolar coupling constants are quite invariant with even major covalent electronic perturbations.[23]

(8) Viscosity measurements were also performed (at room temperature) which suggest that the total viscosity change which occurred during the titrations is only ~5%.

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Table I. Rotational Correlation Times for $^2H$ in AK·AMPPCP Complexes

| complex | line width (Hz) | $\tau_c$ (ns) |
|---|---|---|
| AK·AMPPCD$_2$P | 367 ± 15 | 6.5 ± 0.5[a] |
| AK·MgAMPPCD$_2$P | 660 ± 50 | 16 ± 1[a] |
| AK·[8-$^2$H]AMPPCP | 1250 ± 150 | 27 ± 4[a] |
| AK | | 75[b] |

[a] Calculated from line widths as described in ref 6 and 7. [b] Calculated from the Stokes-Einstein equation assuming that AK is a rigid sphere.[10]

at the same magnetic field (46.1 MHz) and from line width measurements at a different magnetic field (78.7 MHz).

The $\tau_c$ values reported in Table I indicate that the adenine ring of bound AMPPCP is motionally rigid[9] and is close to the overall $\tau_c$ of AK.[10] The $\beta$-$\gamma$ region of the phosphonate chain possesses considerable local mobility.[9] This motional freedom is greatly reduced upon the binding of $Mg^{2+}$ (ATP and MgATP have been shown[11] to bind to the same site of AK).

While it is difficult to quantitatively dissect the overall AK·ATP binding energy to contributions by the various local segments of ATP/MgATP[2,12] qualitative observations can be made regarding the relationship between local binding energy and local motion. $Mg^{2+}$ appears to make very little contribution to the overall binding energy since MgATP and ATP bind to AK with nearly equivalent affinities, as do MnPPPi and PPPi.[13] However, our $^2H$ NMR results with AMPPCP indicate that $Mg^{2+}$ does induce a significant increase in the $\tau_c$ of the phosphate chain.[3] Furthermore, the adenine ring of bound ATP is held rigidly compared to the triphosphate moiety, but the dissociation constant for adenosine binding to the ATP site of AK has been reported to be greater than 20 mM (compared to the value of 0.1 mM for ATP).[14] Thus, it appears that there is no correlation between local substrate dynamics and local binding energy.

Our results suggest the following important points. (a) Thermodynamically "tight" local binding does not necessarily imply local motional rigidity for the bound substrate (and vice versa). (b) Since AK is known to be more specific for ATP in catalysis than in binding (based on the $k_{cat}$ and $K_m$ values of ATP and its analogues)[13,15] and since the adenine ring is rigid at the ground state, the additional binding energy expressed at the transition state should be used primarily to increase the specificity not to rigidize the adenine ring. (c) Binding of $Mg^{2+}$ may serve, among other functions, to immobilize and properly orient the $\gamma$-phosphate in preparation for the transition state. (d) Although the relatively large local freedom of the triphosphate moiety of AK·ATP has been revealed qualitatively by $^{31}P$ NMR (very narrow signals of bound ATP)[16] and $^{17}O$ NMR (relatively small increases in $\Delta\nu_{1/2}$ upon binding),[17] we have demonstrated that $^2H$ NMR can provide a comparison of relative local motional freedom in a straightforward way.

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(9) Although the $\tau_c$ obtained from $^2H$ NMR are only effective values ($1/\tau_{c,eff} = 1/\tau_{c,overall} + 1/\tau_{c,internal}$),[24] differences in $\tau_{c,eff}$ determined for the various species can be used to compare the local, internal dynamics of the different groups since $1/\tau_{c,overall}$ can be assumed to be similar for the three species since it is dictated by protein motion.

(10) The calculated value of 75 ns presented in Table I is almost certainly a great overestimation of this value since the calculation does not take the dynamic nature of proteins into account.

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(25) For MgAMPPCP, the points where the fraction bound exceeds 0.5 were not shown since at such low concentrations AMPPCP may not be fully complexed with $Mg^{2+}$, probably due to competition by other nonspecific binding. As a consequence some of these points fell below the plotted line and were difficult to reproduce.

## Synthesis and Structural Characterization of a Thiolate Coordination Complex of a Mixed-Valence Mo(V)/Mo(VI) Polyoxomolybdate, $[Mo_{10}O_{28}(SCH_2CH_2O)_2(HOCH_3)_2]^{4-}$, and of Its Decomposition Product $[Mo_4O_6(SCH_2CH_2O)_5]^{2-}$

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Coordination compounds of isopolyoxomolybdate anions are of fundamental chemical interest as models for the interactions of substrates with metal oxide surfaces.[1] As a consequence of the development of polyoxoanions soluble in organic solvents, the synthetic chemistry of isopolyoxomolybdates has been extended beyond Bronsted acid-base chemistry to include a variety of complexes incorporating oxygen-[2-7] or nitrogen-containing[8-15] organic ligands. However, the coordination chemistry of polyoxometalates in general with sulfur-containing ligands remains undeveloped,[16] with the exceptions of a number of sulfido-polyoxometalate species[17,18] and a single example of a polyoxomolybdate ligated to an organodisulfide group.[19]

The notable absence of thiolate-containing isopolymolybdate

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