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The Entropic and Enthalpic Contributions to Force-Dependent Dissociation Kinetics of the Pyrophosphate Bond

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Supporting Information

ABSTRACT: We report quantum-chemical calculations of the activation free energy of solvolysis of the pyrophosphate bond in a conformationally flexible reactant coupled to a constraining potential. The results reveal a significant contribution of conformational entropy to the force-dependent kinetics of even a fairly small reactant, suggesting that accurate predictions or molecular interpretation of localized reaction kinetics in stretched polymers may require explicit consideration of their force-dependent conformational heterogeneity. We further show that modeling the conformational space of the reactant and the transition state as collections of overlapping harmonic wells accurately predicts the force-dependent activation free energy up to 2 nN without detailed quantum-chemical computations. An estimate of the activation energies is obtained from the minimal (Eyring−Bell−Evans) model using the local coordinate common to all nucleophilic displacement reactions.

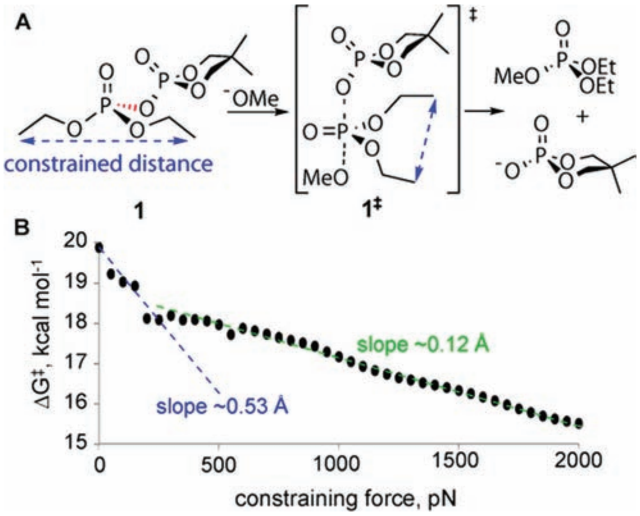

Figure 1. (A) Reaction scheme and (B) calculated activation free energy as a function of the constraining force.

Understanding the molecular and chemical responses of polymeric materials to mechanical loads is one of the grand challenges of physical sciences in the 21st century.[1−3] The conceptual and technical complexity of the very large range of time and length scales that govern the dynamics of such systems may be overcome by treating processes at different scales with distinct formalisms (e.g., continuum mechanics, statistical mechanics, chemical kinetics) and using proper coupling to yield a coherent description of the overall dynamics.[4] Within this approach, a localized reaction in a polymer in elongational flow or in a bulk material under mechanical load can be modeled as a reactive moiety whose single internuclear distance is constrained with a (usually harmonic) potential.[5−7] To date, quantum-chemical tests of this model have focused on small, rigid reactants that exist as a single conformer or have been limited to such strong forces that only the longest conformers were populated.[8−13] This neglect of conformational heterogeneity of canonical ensembles is an oversimplified description of localized reactions in long flexible polymers in elongational flows,[14] single-molecule force experiments,[5] and macroscopically loaded materials.[15]

Here we report density functional theory (DFT) calculations of the activation free energy for methanolysis of the pyrophosphate bond in reactant **1** coupled to an infinitely soft, massless constraining potential (Figure 1). Nucleophilic dissociation of the pyrophosphate bond is the primary chemomechanical energy-transducing mechanism in living systems.[16] We chose to study pyrophosphate **1** because (1) it has significant conformational flexibility both along (through the EtOPOPOEt moiety) and orthogonal to [through the OP(═O)(*c*-$O_2(CH_2)_2CMe_2$ moiety] the constrained molecular axis and (2) the P atom of the side chain is sterically blocked from undergoing ligand displacement, simplifying experimental tests of the computational and theoretical results presented here. The calculations (1) demonstrated a significant entropic component of the difference in the activation free energies of strain-free and constrained conformational ensembles and (2) validated a simple statistical-mechanics model that predicts force-dependent kinetics only from the data on unconstrained reactive sites.

Calculations at the B3LYP/6-31+G* and B3LYP/6-311++G** levels using SMD[17−22] as the solvent model identified 22 and 17 thermally accessible conformers of the unconstrained ground state (**1**) and transition state (**1**‡), respectively [Table S1 in the Supporting Information (SI)].[23] All of the optimized conformers of **1**‡ had a classical S_N2 geometry[24] with the three O atoms of the spectator ligands being nearly coplanar with the electrophilic P (ensemble-averaged sum of the three O−P−O angles: 353°), an elongated scissile P−O(P) bond (ensemble-averaged elongation of 0.091 Å), and a long forming P−O(Me) bond (2.53 Å). We found no energy minima corresponding to

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five-coordinate P species (associative intermediates). All dissociative intermediates with a three-coordinate P were significantly higher in energy than the S_N2 transition states. Reaction path calculations confirmed that these transition states were linked directly to the reactant and the product (Figure S1 in the SI). These findings are consistent with the current consensus that simple pyrophosphates solvolyze by an S_N2 mechanism.[25]

The computed strain-free activation free energies of methanolysis at 298 K were 19.9 and 20.0 kcal/mol with the 6-31+G* and 6-311+G** basis sets, respectively. The experimental activation free energy of methanolysis of **1** has not been reported, but that of tetraethylpyrophosphate (TEPP) is 17.5 ± 0.8 kcal/mol at 298 K according to our measurements. The lower symmetry of **1** increases its activation free energy relative to that of TEPP (which has two equivalent electrophilic P atoms) by $\sim RT \ln 2 = 0.4$ kcal/mol. We therefore conclude that B3LYP/6-31+G* with SMD is a reasonable model for the reaction of **1**.

We calculated the activation free energy of methanolysis of **1** coupled to an infinitely soft, massless harmonic constraining potential, $\Delta G^{\ddagger}$, using eq 1, in which k is the Boltzmann

$$\Delta G^{\ddagger}_{\{c\}}(f) = -kT \ln \frac{\sum_{i=1}^{n^{\ddagger}(f)} e^{-\frac{E_i^{\ddagger}(f)+TC_i^{\ddagger}(f)-f\times q_i^{\ddagger}(f)}{kT}}}{\sum_{j=1}^{n(f)} e^{-\frac{E_j(f)+TC_j(f)-f\times q_j(f)}{kT}}} \qquad (1)$$

constant; $E_i(f)$, $TC_i(f)$, and $q_i(f)$ are the electronic energy, its thermodynamic correction, and the constrained distance of conformer i in equilibrium with force f, respectively; and the summations are over all of the thermally accessible conformers, whose numbers $n(f)$ and $n^{\ddagger}(f)$ vary with f. The infinite compliance of the constraining potential ensures that in mechanical equilibrium, the restoring force of each constrained conformer of **1** and $\mathbf{1}^{\ddagger}$ is identical,[5,6] irrespective of their structural differences. We obtained $E_i(f)$ from a relaxed potential energy scan of each conformer and $TC_i(f)$ in the ideal gas/harmonic oscillator/rigid rotor approximation from analytical Hessians of constrained geometries.[26] The application of this statistical-mechanics formalism is justified because such constrained geometries are stationary points of a molecule coupled to an infinitely soft massless constraining potential.[6] The term $f \times q_i(f)$ is the strain energy of the constraining potential relative to the reference value $f^2 \times \lambda_c$, where λ_c is the harmonic compliance of the constraint.

We confirmed that constraining **1** does not change its reaction mechanism at forces up to 2 nN. At all forces, the free energies of the constrained three-coordinate intermediates significantly exceeded those of the S_N2 transition states (Figure S2). Reaction path calculations with the constraining potential implemented within the ONIOM scheme revealed no reaction intermediates (Figure S1c).

The calculated $\Delta G^{\ddagger}$ as a function of the constraining force f (Figure 1B) manifests two distinct regimes: a rapid decrease of $\Delta G^{\ddagger}$ for $f < 300$ pN ($\Delta\Delta G/N_A\Delta f \approx 0.53$ Å) followed by a much less force-sensitive regime for $f > 300$ pN ($\Delta\Delta G/N_A\Delta f \approx 0.12$ Å). The two regimes are attributable to the predominance of the entropic ($f < 300$ pN) and enthalpic ($f > 300$ pN) contributions. The unconstrained conformational ensemble of **1** is made up of many more conformers with short $H_3C\cdots CH_3$ distances in comparison with the ensemble of $\mathbf{1}^{\ddagger}$, whose higher coordination number of P destabilizes gauche configurations of the ethyl chains (Figure 2). Annihilation of these compact conformers for $f < 300$ pN increases the free energy of **1** relative to $\mathbf{1}^{\ddagger}$. For $f > 300$ pN, conformers with an all-anti configuration of the EtOPOEt chain dominate, and the activation free energy decreases primarily because of the lower strain energy of the constraining potential coupled to the longer transition state $\mathbf{1}^{\ddagger}$ relative to ground state **1**.

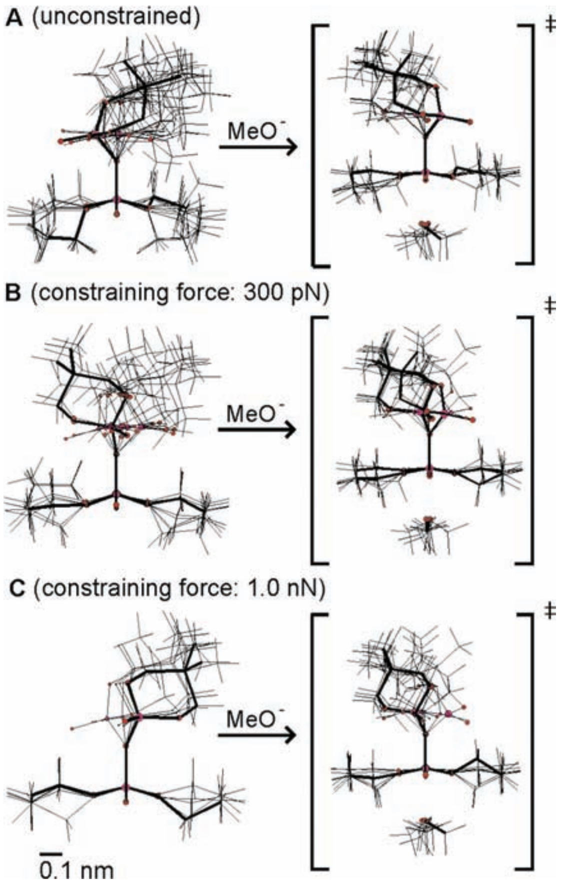

Figure 2. Superposition of the optimized geometries of conformers of **1** and $\mathbf{1}^{\ddagger}$ (A) without constraint and (B, C) coupled to an infinitely soft massless constraining potential with a force of (B) 300 pN and (C) 1 nN at the terminal C atoms of the ethyl groups. P and O atoms are shown as red dots. The thickness of the lines is proportional to the mole fraction of each conformer in the ensemble. The considerably more limited range of geometries of both the ethyl groups and the side chain in the coupled system should be noted. The structures were overlaid by minimizing the root-mean-square deviation of the PO_4 core, with the P atom at the origin.

A unique value of the force formalism lies in its potential to allow usefully accurate extrapolations of localized reaction rates in stretched polymers from the readily available structural and reactivity data on individual isolated, strain-free monomers. For the reaction of **1**, such an extrapolation to the coupled system is possible with a simple statistical-mechanics model that approximates the conformational landscape of **1** and $\mathbf{1}^{\ddagger}$ as a collection of overlapping energy wells, each defined by eq 2, in

$$G_i(f) = G_i^o + f^2\lambda_c - N_A f\left(q_i^o + \frac{f}{2}\lambda_i^o\right) \qquad (2)$$

which q_i^o is the equilibrium internuclear distance of conformer i at which it is coupled to the constraining potential, λ_i^o is the harmonic compliance of this distance, and N_A is the Avogadro constant. Equation 2 applies for $f < f_i^{\max}$, where $f_i^{\max}$, the force above which conformer i ceases to exist, is defined by the parameters of strain-free conformers (eq S2 in the SI). Within this model, the force-dependent activation free energy is given by eq 3, where only conformers with $f_i^{\max} > f$ are counted.

$$\Delta G^{\ddagger}_{\{c\}}(f) = -RT \ln \frac{\sum_{i=1}^{n^{\ddagger}(f)} e^{-\frac{G_i^{o\ddagger} - N_A f\left(q_i^{o\ddagger} + \frac{f}{2}\lambda_i^{o\ddagger}\right)}{RT}}}{\sum_{j=1}^{n(f)} e^{-\frac{G_j^{o} - N_A f\left(q_j^{o} + \frac{f}{2}\lambda_j^{o}\right)}{RT}}} \qquad (3)$$

Equation 3 requires no fitting parameters or parameters from constrained structures. It reproduces the calculated $\Delta G^{\ddagger}$ values accurately up to ~1 nN (blue line in Figure 3). For stronger forces, the anharmonicity of the few surviving conformers becomes significant. Replacing the $G^{o} - N_A f(q^{o} + f\lambda^{o}/2)$ term for the *longest* (all-anti) conformer of both the reactant and the transition state with the force-dependent relative free energy of the coupled system, $G(f) - fq(f)$, derived from a relaxed potential energy scan, yields usefully accurate predictions up to >2 nN (green line). Thus, the harmonic compliances and equilibrium geometries of all of the (unconstrained) conformers of **1** and **1**‡ constitute the minimum set of data needed to obtain usefully accurate force-dependent activation free energies for the methanolysis of **1** up to 1 nN, and adding the results of constrained energy scans for the longest (all-anti) conformers of **1** and **1**‡ yields accurate predictions up to 2 nN.

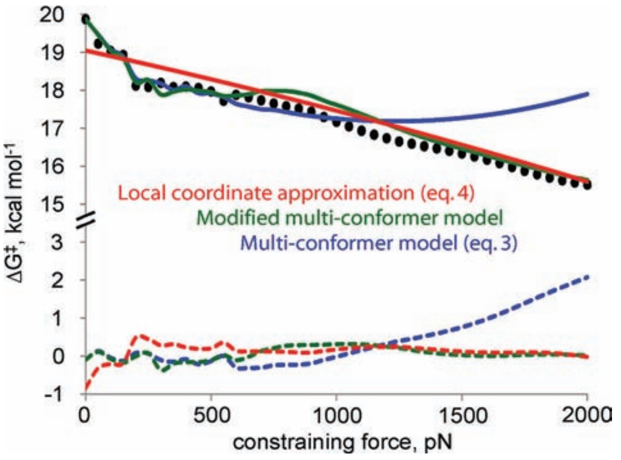

Figure 3. Quantum-mechanically calculated (black ●) and predicted (solid lines) activation free energies for hydrolysis of pyrophosphate coupled to a massless, infinitely soft constraining potential and the differences between the predicted and exact values (dashed lines).

In certain cases, optimization of every thermally accessible conformer of a reactive site can be impractical, and a fast, order-of-magnitude estimate may be preferable (e.g., at the initial stages of designing stress-responsive monomers).[2] The widely used Eyring–Bell–Evans ansatz,[27–29] which postulates a direct proportionality between the activation free energy and the constraining force, is generally considered to have no predictive capabilities,[30,31] since it does not define the proportionality constant in molecular terms. We previously suggested[7,6] that this constant can be approximated by a judiciously chosen local internuclear distance, q_l, which is the same for all reactions of the same mechanism (eq 4). In nucleophilic displacement reactions [I (S_N2), D (S_N1), and A mechanisms], q_l is defined by the pair of atoms that connect the electrophilic atom to the polymer backbone. In model reactant **1**, q_l is thus the (Et)O···O(Et) separation, which is, incidentally, orthogonal to the scissile bond.

$$\Delta G^{\ddagger}_{\{c\}}(f) \approx \Delta G^{\ddagger}_{o} - N_A f\left(q_l^{o\ddagger} - q_l^{o}\right) \qquad (4)$$

The red line in Figure 3 shows the performance of eq 4 using parameters for the longest (all-anti) conformers of **1** and **1**‡. Because of the conformational flexibility of the side chain, several distinct conformations of **1** and **1**‡ in which the EtOPOPOEt chain is all-anti exist, but all such conformers have very similar Δq_l values. Equation 4 has the largest error at low forces, which is expected as it neglects entropic factors.

The two distinct regimes of $\Delta G^{\ddagger}(f)$ predicted here (Figure 1) are often observed in single-molecule measurements of localized bimolecular reactions in stretched polymers.[7,32] Because of the lack of experimental methods to identify the molecular origin of such biphasic behavior,[2,30,33] it is traditionally ascribed to changes in the reaction mechanism.[7] The data presented here suggest that an alternative—conformational entropy—must be considered. The continued development of variable-temperature single-molecule force experiments[34] could allow measurements of the enthalpy and entropy of activation of localized reaction in stretched polymers as a function of the constraining force with sufficient accuracy to differentiate these two potential causes of nonlinearity.

Molecular interpretation of such future measurements requires an understanding of how $\Delta G^{\ddagger}(f)$ changes with the length of the constrained distance and the chemical composition of the *nonreactive* parts of the constrained molecule. Such data would be available by repeating the calculations in this paper for a series of homologous pyrophosphates whose backbones are systematically changed from C_2H_5 in **1** to longer alkyls (C_nH_{2n+1}) to ethers [$(CH_2CH_2O)_nCH_3$], etc. We suggested[6] that above a certain value of n, $\Delta G^{\ddagger}(f)$ is independent of n or the chemical composition of the backbone, but at present we cannot predict this minimal n for an arbitrary reaction. The very large number of conformers of molecules with $n > 2$ makes DFT calculations of their ensembles untenable.[7] The data presented here allow systematic benchmarking of the accuracy of semiempirical and mixed-level methods,[26,35] which are less resource-intensive and may be suitable for calculations of constrained ensembles of larger reactants needed to extrapolate computed $\Delta G^{\ddagger}(f)$ to the limit of an "infinitely" long constrained backbone of arbitrary chemical composition.[7]

Although no experimental technique yet exists to validate the $\Delta G^{\ddagger}(f)$ values calculated in this work, a molecular force probe[7,36] potentially allows the relationship between the restoring force of a *local* coordinate and the change in the activation free energy (red line, Figure 3) to be tested experimentally. Unlike its microscopic counterparts, which require atomistically intractable polymers, a molecular force probe can be used to control the restoring force of a local coordinate (such as the O···O distance of the reactive PO_4 center) in nonpolymeric substrates through molecular design. Measuring the activation free energy of hydrolysis across a series of properly designed, increasingly strained macrocyclic pyrophosphates should enable the experimental $\Delta G^{\ddagger}$ to be correlated with the calculated differential strain (and hence the restoring force) of the O···O coordinate of the PO_4 site in each macrocycle. Such experimental tests of the results reported here are underway in our group.

In summary, the results presented here (1) highlight the importance of conformational entropy in the kinetics of even fairly small reactants coupled to a constraining potential and

(2) define the models and minimum data needed to estimate $\Delta G^{\ddagger}(f)$ with various levels of accuracy. We have shown that modeling the molecular conformational space as a collection of harmonic energy wells adequately reproduces the computed activation free energies up to ~1 nN. A minimal model based on the Eyring–Bell–Evans ansatz requires optimized longest (all-anti) conformers to yield reasonably accurate $\Delta G^{\ddagger}(f)$ values but only for the properly chosen local coordinate as the proportionality constant.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Computational procedures; coordinates, energies, and frequencies of the optimized structures; results of IRC calculations; and energies of three-coordinate intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

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