The elucidation of RNA reaction mechanisms ...

... has a wide range of implications, from the origin of life to biotechnology. In their Communication on page 647 ff., K.-Y. Wong, J. A. Piccirilli, M. E. Harris, D. M. York, et al. report a joint theoretical and experimental study on kinetic isotope effects in models of RNA cleavage transesterification. The characteristics of the mechanism and the transition states are distinctly different in the reaction with the native compound relative to two thio-substituted analogues.
Characterization of the Reaction Path and Transition States for RNA Transphosphorylation Models from Theory and Experiment**

Kin-Yu Wong,* Hong Gu, Shuming Zhang, Joseph A. Piccirilli,* Michael E. Harris,* and Darrin M. York*  

The elucidation of the chemical mechanisms whereby biological molecules control, regulate, and catalyze phosphoryl-transfer reactions has profound implications for processes such as transcription, energy storage and transfer, cell signaling, and gene regulation.[1,2] In particular, the catalytic properties of RNA have applications in the design of new biotechnologies, and are also implicated in the evolutionary origins of life itself.[3] The characterization of the transition state for any given reaction is of primary importance in understanding the reaction mechanism. Kinetic isotope effects (KIEs) offer one of the most powerful and sensitive tools for a detailed mechanistic interpretation based on the experimental values for comparison in Table 2. The agreement between the theoretical and experimental results allows KIE values, together with primary isotope effect measurements from previous reports,[12,13] represent a comprehensive characterization of isotope effects for a native (unmodified) RNA system.

Scheme 1 illustrates the general mechanism for the reverse, diaionic, in-line methanolysis of ethylene phosphate, which is a model for base-catalyzed RNA phosphate transesterification. The phosphoryl oxygen positions are labeled in accordance with their RNA counterparts. The free energy profiles for Scheme 1 were determined through density-functional quantum mechanical/molecular mechanical (QM/MM) simulations in explicit solvent (Figure 1, top graph).[14–17] These simulations take into account the dynamic fluctuations of the solute and the degrees of freedom of the solvent in determining the free energy profiles. In addition, the adiabatic reaction energy profiles with solvation effects treated implicitly with a polarizable continuum model (PCM)[18] were determined (Figure 1). The PCM model was specifically calibrated for the native model compound, as well as the 3'- and 5'-thio-substituted compounds (referred hereafter as S3' and S5', respectively; Figure 1). The S3' and S5' compounds are models for the corresponding chemically modified RNAs that serve as valuable experimental probes of phosphoryl transfer mechanisms catalyzed by ribozymes.[19]

For example, the S3' substitution in the HDV ribozyme serves as an enhanced leaving group that suppresses the deleterious effect of a mutation of a critical cytosine residue, which has been interpreted to support its role as a general acid catalyst.[20]

The energy values for stationary points of the reactions with the native and thio-substituted compounds are shown in Table 1. By using our recently developed ab initio path-integral method, which is based on Kleinert’s variational perturbation theory,[17,21–23] we also calculated the KIE values. These KIE values are shown along with the most relevant experimental values for comparison in Table 2. The agreement between the theoretical and experimental results allows for a detailed mechanistic interpretation based on the theoretical models.[7,8]

All of the profiles calculated in this study correspond to associative (or concerted) mechanisms, characterized by an initial nucleophilic attack, as is typical for phosphate diesters.[24] The departure of the leaving group can occur at the same time as the nucleophilic attack (as in the reaction with the S3' compound) or can occur in a stepwise fashion, which...
formation and a shallow, metastable intermediate. In both cases, TS2 is rate-controlling. A comparison of the activation free energy values calculated from the QM/MM simulations and from the adiabatic reaction profiles (which include zero-point and thermal corrections; Table 1) indicates that TS1 has very similar activation energy in the two profiles (18.8 kcal mol\(^{-1}\) and 18.6 kcal mol\(^{-1}\), respectively). In contrast, TS2 is 3.1 kcal mol\(^{-1}\) higher in energy in the QM/MM simulation. The rate-controlling TS2 has considerable “late” character, in which cleavage of the exocyclic P–O\(^{\prime}\) bond is advanced (Table 1).

The density-functional QM/MM free energy profile\cite{24,25} and the PCM adiabatic reaction profile for the reaction of the native compound are very similar (Figure 1, top). Both profiles show an associative mechanism and have distinct TS1 and TS2 transition states, which are separated by a shallow, metastable intermediate. In both cases, TS2 is rate-controlling. A comparison of the activation free energy values calculated from the QM/MM simulations and from the adiabatic reaction profiles (which include zero-point and thermal corrections; Table 1) indicates that TS1 has very similar activation energy in the two profiles (18.8 kcal mol\(^{-1}\) and 18.6 kcal mol\(^{-1}\), respectively). In contrast, TS2 is 3.1 kcal mol\(^{-1}\) higher in energy in the QM/MM simulation. The rate-controlling TS2 has considerable “late” character, in which cleavage of the exocyclic P–O\(^{\prime}\) bond is advanced (Table 1).
Table 2: Primary KIE values on the 2' nucleophile ($^{18}k_{Nu}$) and the 5' leaving group ($^{18}k_{Lg})$, and secondary KIE values on O'' ($^{18}k_{O''})$ and O''' ($^{18}k_{O'''}$ in aqueous solution for TS1 and TS2, along with the most relevant available experimental results for comparison.[5]

<table>
<thead>
<tr>
<th>Reaction</th>
<th>TS1</th>
<th>TS2</th>
<th>Expt</th>
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<tbody>
<tr>
<td>native</td>
<td>1.017</td>
<td>1.006</td>
<td>0.968</td>
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<tr>
<td>native</td>
<td>1.004</td>
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<tr>
<td>$^{18}S''$</td>
<td>1.004[10]</td>
<td>1.004[10]</td>
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[a] Temperatures used in calculations are identical to those used in the experiments that are being compared: 37°C for reactions with the native compound, and 80°C for reactions with S' and S'' compounds. All primary KIE values are calculated using the second order Kleinert’s variational perturbation theory with decoupled instantaneous normal coordinate approximation, and the secondary KIE values are calculated using the Bigeleisen equation in the decoupled, rigid-rotor, harmonic-oscillator approximation.[27,28] Values in bold represent the rate-controlling transition state of the reaction with the native compound. These calculations are from a combination of the stabilizing electronic effects of the soft sulfur atom in the equatorial position of the pentavalent transition state that is able to help delocalize charge, and a partial alleviation of ring strain through the longer P-O' bond. Solvation effects are also present, but are less pronounced than at the nonbridging positions which carry a formal negative charge.

The anharmonic contributions to all the calculations of the primary KIE values which were estimated by using our ab initio path-integral method[7] are listed in Table 2. These values are in good agreement with the experimental results. The term “good agreement” refers to agreement within the resolution of the KIE values as being large inverse (less than 0.97), inverse (0.97–0.99), near unity (0.99–1.01), normal (1.01–1.03), and large normal (greater than 1.03). In the experiment, the model reaction for RNA transesterification is the base-catalyzed 2'-O-transphosphorylation of the RNA dinucleotide 5'-UpG-3.[29] Since both deprotonation of the 2'-OH group and formation of the P-O' bond will contribute to the KIE values measured in the experiments, the values reported here for $^{18}k_{Nu}$ and $^{18}k_{Lg}$ (at the infinite pH limit) do not include the equilibrium isotope effect on alcohol deprotonation. The inverse KIE value of $^{18}k_{Nu}$ (0.981) and the large normal KIE value of $^{18}k_{Lg}$ (1.034) measured experimentally[23] for TS2 agree well with the KIE values calculated for TS2 (0.968 and 1.059; Table 2). In contrast to the rate-controlling TS2, the KIE value calculated for $^{18}k_{Nu}$ based on TS1 is normal (1.017), and the KIE value calculated for $^{18}k_{Lg}$ is close to unity (1.006). This finding supports the interpretation that the KIE values measured for the reaction with the native compound (UpG transphosphorylation) are consistent with a rate-controlling transition state that is characterized by an almost fully formed P-O' bond and an almost fully cleaved P-O' bond (Table 1 and Figure 2).
the signature of the TS1 transition state (Table 2 and Figure 2). This experimental \(^{18}k_{\text{Nu}}\) value\(^{[13]}\) reflects a reaction from the ground state in which the 2-oxygen atom is not deprotonated, and includes a somewhat large normal contribution of 2–4 % from the equilibrium isotope effect on alcohol deprotonation. Accordingly, the KIE values were calculated including this contribution. As in the case for the reaction with the native compound, the secondary KIE values for the reaction with the S\(^5\) compound are close to unity (Table 2).

The sulfur substitution at the S\(^5\)-position results in a reaction profile that is unimodal, and the activation energy is lower than in the reaction profiles of the native and S\(^3\) compounds (Figure 1, bottom, and Table 1). Although sulfur is less apicophilic than oxygen in pentavalent phosphorus compounds,\(^{[35]}\) this effect is not predicted to be large in the present calculations, because the free energy of formation of TS1 in the reaction with the S\(^5\) compound is only 2 kcal mol\(^{-1}\) lower than for the reaction with the native compound. The main contribution to the differences in the reaction profiles of the native and S\(^5\) compounds is derived from the thiolate being an enhanced leaving group, relative to the methoxide anion (the p\(_K\) value of primary alcohols is typically around 5–6, units higher than the corresponding thiols). The difference in leaving group leads to the elimination of TS2 from the profile of the reaction, and a shift towards an early transition state character (a smaller P–O\(^{-}\) bond order). The value of \(\Delta\text{bond}\) in Table 1 and Figure 2 for the transition state is \(-0.02\ \text{Å},\) but this value reflects the fact that the bond length of P–S\(^5\) is approximately 0.5 Å longer than the P–O\(^{-}\) bond. The rate-controlling barrier for the reaction with the S\(^5\) compound is approximately 16.6 kcal mol\(^{-1}\), which is the smallest of the reaction models studied here. The size of this barrier suggests that this substitution reaction will have the fastest rate. This result is consistent with experimental studies of S\(^5\)-substituted reaction models.\(^{[13,36,37]}\)

The primary KIE values predicted for both \(^{18}k_{\text{Nu}}\) (1.042) and \(^{34}k_{\text{Nu}}\) (1.002) for the reaction with the S\(^5\) compound (Table 2) are large normal and close to unity, respectively, and are in agreement with the experimental results \(^{18}k_{\text{Nu}}\) (1.025; \(^{34}k_{\text{Nu}}\) : 1.001) for the cyclization of \(m\)-nitrobenzyl ribonucleoside phosphodiester with S\(^5\) substitution.\(^{[13]}\) This agreement supports the notion that the rate-controlling transition state for the reaction with the S\(^5\) compound is TS1.

The secondary KIE values for the nonbridging phosphor oxygens in the reaction with the native UpG dinucleotide were measured (Table 2). The present model system involves an associative reaction with a diionic phosphate diester and the added complexity of coupling between the vibrational modes of the ring in the formation of the transition state. The KIE values which were measured for the nonbridging phosphor oxygen positions were very close to unity (0.999). Within the error ranges, these KIE values are in good agreement with the KIE values of 1.003–1.005 which were calculated for the reaction with the native compound (Table 2). Unlike the calculated differences between the primary KIE values, the secondary KIE values for the reactions with the native, S\(^3\), and S\(^5\) compounds, were all close to unity.

In conclusion, we have reported the density-functional combined QM/MM simulations and solvated adiabatic reaction profiles for model transphosphorylation reactions of native and thio-substituted RNA. The KIE values were calculated by using our recently developed ab initio path-integral method,\(^{[7,21–23]}\) which takes into account the treatment of internuclear quantum effects. Additionally, we measured the secondary KIE values for the reaction of native UpG. This dinucleotide sequence represents the cleavage site in the HDV ribozyme,\(^{[9,12]}\) and we predicted the secondary KIE values for reactions with thio-substituted analogues. Our results provide an atomic-level picture of the reactions, which includes a detailed characterization of the rate-controlling transition states. These transition states are in agreement with experimental measurements of the rates of reaction and the primary and secondary KIE values. The reaction with the native compound is characterized by a rate-controlling transition state which corresponds to exocyclic bond cleavage of the leaving group. This reaction also has an inverse primary nucleophile KIE value. The reaction with the S\(^5\) compound is characterized by two distinct transition states. The transition state which corresponds to the nucleophilic attack, rather than the departure of the leaving group, gives rise to the large normal primary nucleophile KIE value which was observed in experiments. The reaction with the S\(^5\) compound is concerted, has a unimodal reaction profile with the lowest barrier, and has large normal primary KIE values. Unlike the striking differences between the primary KIE values which were calculated for the reactions with the native and thio-substituted compounds, all of the secondary KIE values are close to unity. Together, these results paint a detailed picture of the reaction mechanisms of model phosphoryl-transfer reactions.

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