

## Hybrid QM/MM Study of Thio Effects in Transphosphorylation Reactions

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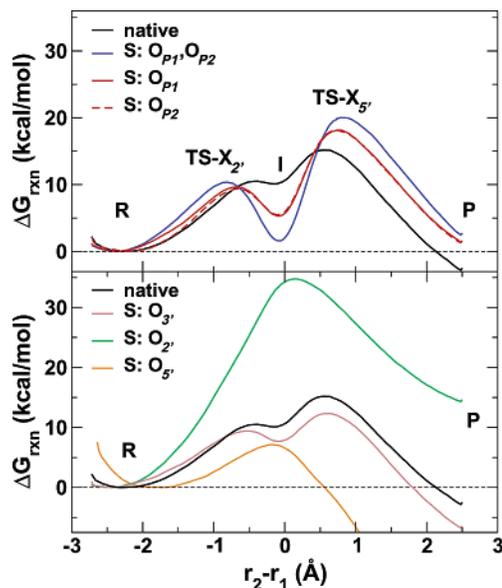
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The discovery that RNA molecules can act as enzymes (ribozymes) has prompted great interest in the study of the molecular mechanisms of their catalytic action. A prototype reaction for RNA catalysis is the transphosphorylation and hydrolysis of phosphodiester bonds that form the anionic backbone linkage in DNA and RNA.<sup>1–6</sup> These systems have been the focus of theoretical studies in the gas phase,<sup>7–10</sup> in solution<sup>11–17</sup> (including *ab initio* studies of the nature of associative and dissociative paths<sup>18–20</sup>), and in enzymatic environments.<sup>21–23</sup> Despite tremendous experimental and theoretical effort, the reaction mechanism for phosphodiester transphosphorylation and hydrolysis is not fully understood.

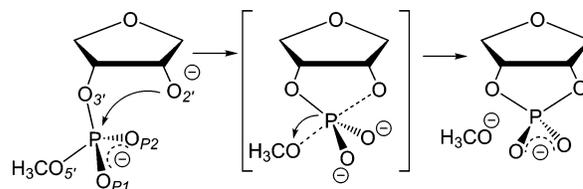
A powerful experimental technique used to probe the mechanistic details of RNA catalysis is the analysis of “thio effects”,<sup>3,24</sup> i.e., the change in reaction rate that occurs when oxygen atoms are substituted by sulfur at selected positions. Theoretical studies can provide valuable aid in the mechanistic interpretation of thio effect experiments. Hybrid quantum mechanical/molecular mechanical (QM/MM) methods, pioneered by Warshel,<sup>25</sup> are particularly powerful tools.<sup>26–28</sup> It would be preferable to employ a high-level *ab initio* quantum model<sup>28–31</sup> in QM/MM studies of RNA catalysis; however, reliable simulations of ribozyme-catalyzed reactions require consideration of a large quantum region and extensive configurational sampling that often necessitates the use of more efficient (e.g., semiempirical) quantum models. The study of transphosphorylation thio effects further requires consideration of *d*-orbitals for P and S atoms to give reasonable transition states and reaction profiles (Supporting Information and Figure 1). An important step toward a detailed understanding of thio effects in ribozymes is to develop quantum models that are sufficiently accurate and efficient to make feasible application to enzymatic systems and to test these models against nonenzymatic reactions in solution that have been well-characterized by experiment and *ab initio* methods.<sup>18–20</sup> In this communication, a *d*-orbital semiempirical QM/MM method<sup>32</sup> is used to study thio effects in the transphosphorylation (methanolysis) of a 2'-ribose, 5'-methyl phosphate-diester (Scheme 1) under basic conditions.

The native (unsubstituted) reaction (Figure 1) proceeds through two transition states corresponding to formation of the P–O<sub>2'</sub> bond (*TS*<sub>O<sub>2'</sub></sub>) and departure of the 5'-methoxy group (*TS*<sub>O<sub>5'</sub></sub>), separated by a kinetically insignificant intermediate. Single and double thio substitutions at the nonbridging phosphate oxygen positions do not alter the nature of the rate-limiting transition state; however, the softer sulfur atoms afford considerable stabilization of the dianionic phosphorothioate intermediate, and raise the barriers of both *TS*<sub>O<sub>2'</sub></sub> and *TS*<sub>O<sub>5'</sub></sub> (Table 1). This is in accord with experimental observations that a stable dianionic intermediate for the native reaction is unlikely and that sulfur substitution at the nonbridging phosphoryl oxygen positions results in the accumula-



**Figure 1.** Reaction profiles showing thio effects for the in-line dianionic mechanism of transphosphorylation (see text). Free energies relative to the reactants are shown as a function of the  $r_2 - r_1$  reaction coordinate ( $r_1$  and  $r_2$  are the  $P \cdots X_{2'}$  and  $P \cdots X_{5'}$  distances, respectively).

### Scheme 1. In-Line Dianionic Mechanism of Transphosphorylation



tion of intermediates with larger rate-limiting barriers, especially in the case of double substitutions.<sup>2,3</sup>

Thio substitutions at 2' and 5' positions have a profound effect on the free energy profile, causing it to be dominated by a single transition state. The 5'-substitution results in a shift of the rate-limiting transition state toward *TS*<sub>O<sub>2'</sub></sub> and lowers the barrier relative to the native reaction. This results from the increased stability of the 5' thiolate leaving group (thiol *pK<sub>a</sub>* values are typically 5 units lower than the corresponding alcohols) and is consistent with the Hammond postulate.<sup>33</sup> Alternately, the 2'-substitution results in a *TS*<sub>O<sub>5'</sub></sub>-type transition state with an increased barrier relative to the native reaction. These results are consistent with experimental and theoretical work that suggests these reactions proceed through a single transition state with greatly reduced rate.<sup>2,3</sup> One must bear in mind that experimental data may be consistent with multiple mechanistic interpretations<sup>6</sup> and that theoretical studies based on gas-phase models are likely irrelevant for the reactions in solution.

The 3'-thio substitution results in a decreased rate-limiting barrier with a shoulder reminiscent of *TS*<sub>O<sub>2'</sub></sub> in the native reaction. The

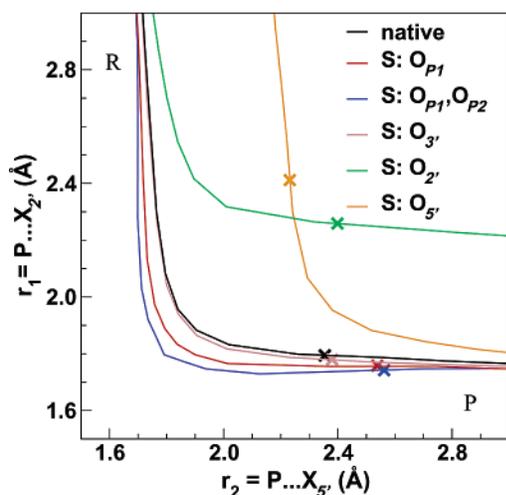
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**Table 1.** Comparison of Thio Effects for the In-Line Dianionic Mechanism of Transphosphorylation in Solution<sup>a</sup>

reaction	relative free energy (kcal/mol)				rate-limiting TS		
	$TS_{X2'}$	I	$TS_{X5'}$	P	TS type	$r_1$ (Å)	$r_2$ (Å)
native	10.5	10.1	15.2	-2.8 <sup>b</sup>	$TS_{X5'}$	1.79	2.35
S: $O_{P1}$	9.6	5.3	18.2	1.3 <sup>b</sup>	$TS_{X5'}$	1.76	2.54
S: $O_{P2}$	9.4	5.5	18.1	1.3 <sup>b</sup>	$TS_{X5'}$	1.75	2.48
S: $O_{P1}, O_{P2}$	10.3	1.6	20.0	2.5 <sup>b</sup>	$TS_{X5'}$	1.74	2.56
S: $O_{3'}$	9.4	7.7	12.3	-6.7 <sup>b</sup>	$TS_{X5'}$	1.78	2.38
S: $O_{2'}$	-	-	34.8	14.3 <sup>b</sup>	$TS_{X5'}$	2.26	2.40
S: $O_{5'}$	7.1	-	-	-27.4 <sup>b</sup>	$TS_{X2'}$	2.41	2.23

<sup>a</sup> Relative free energies (kcal/mol) with respect to reactants at 300 K along the reaction coordinate for the dianionic in-line attack mechanism of sugar-phosphates and sugar-phosphorothioates in solution. Also depicted are the average values for the average  $r_1 = P \cdots X_{2'}$  and  $r_2 = P \cdots X_{5'}$  distances at the rate-limiting transition state (see text). <sup>b</sup> Data for product was taken from last umbrella sampling point along the reaction coordinate.

**Figure 2.** Plot of the average values of  $r_1$  and  $r_2$  for each umbrella sampling window. The rate-limiting transition state is labeled by an 'X'.

3'-thio substitution under basic conditions results in RNA hydrolysis rates that are 2000-fold increased over the pH range 10–14.<sup>3</sup> The present work suggests the equatorial sulfurs have both stabilizing electronic and destabilizing solvation effects on the rate-limiting transition state. The nonbridging  $O_{P1}$  and  $O_{P2}$  atoms carry the majority of the dianionic charge and are particularly sensitive to substitution of larger sulfur atoms that result in less favorable solvation. The  $O_{3'}$  atom carries much less charge, and substitution by sulfur has an overall stabilizing electronic effect.

The stepwise/concerted nature of the reactions and degree of association/dissociation is illustrated in Figure 2. The reactants and products correspond to the upper left and lower right regions of the plot, respectively. An associative pathway is one that proceeds through the lower left region (initial decrease of  $P \cdots X_{2'}$ ), and a dissociative pathway would proceed through the upper right region (initial increase of  $P \cdots X_{5'}$ ). A concerted path proceeds along the diagonal. All of the transphosphorylation reactions in Figure 2 are

stepwise associative in nature, regardless of thio substitution. Thio substitution of the nonbridging phosphoryl oxygens results in an increased degree of association relative to the native reaction. The 2'- and 5'-thio substitutions are also associative, but the rate-limiting step is characterized by the formation/cleavage of the P–O bond rather than the P–S bond.

The results presented here provide an important step toward the development and application of new hybrid QM/MM methods that, combined with experiment, may provide a detailed picture of the molecular mechanisms of RNA catalysis.

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**Supporting Information Available:** Computational methodology and validation of MNDO/d Hamiltonian (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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