Pseudorotation of Natural and Chemically Modified Biological Phosphoranes: Implications for RNA Catalysis

Carlos Silva López,[b, c] Olalla Nieto Faza,[b]
Brent A. Gregersen,[a] Xabier Lopez,[c]
Angel R. de Lera,[b] and Darrin M. York*[a]

1. Introduction

Biological phosphates form the anionic backbone linkage in DNA and RNA and play a central role in the regulation of cellular processes including signaling, respiration, replication, and translation.[1] Consequently, the study of the chemistry of biological phosphates is an area of great importance.[2–5] Of particular interest are the mechanisms by which RNA can catalyze fairly complicated reactions[6–8] such as the transphosphorylation and hydrolysis of phosphodiester bonds. A useful experimental strategy to probe the catalytic mechanisms of RNA enzymes is the study of thio effects: changes in the reaction rate that occur upon substitution of key phosphate oxygen atoms with sulfur atoms.[9, 10] Kinetic analysis of thio effects provides insight into the specific role that these oxygen positions play in catalysis.[11] Theoretical methods are powerful tools to aid the interpretation of kinetic data through characterization of the structure and energetics of transition states and intermediates along competing reaction paths.[12]

The dominant reaction path for transphosphorylation in RNA[2] (Scheme 1) proceeds via an in-line attack of an activated 2′-hydroxy group of the RNA sugar ring on the reactive phosphate group to produce a pentavalent phosphorane transition state or intermediate, followed by the cleavage of the P-O$^{5′}$ bond to produce a 2′,3′-cyclic phosphate. Experimental and theoretical data suggest a dianionic oxyphosphorane transphosphorylation intermediate is kinetically indistinguishable from a transition state and is too short-lived to undergo other processes such as protonation or pseudorotation.[2, 3] As the pH is lowered, acid-catalyzed migration products begin to emerge,[4] which result from pseudorotation of a singly or doubly protonated phosphorane intermediate (Scheme 2).

The ratio of products resulting from phosphate hydrolysis/transphosphorylation and isomerization (migration) and their pH dependence involve a balance between the endo- and exo-

[a] B. A. Gregersen, Prof. D. M. York
Department of Chemistry, University of Minnesota
207 Pleasant St. SE, Minneapolis, MN 55455–0431 (USA)
Fax: (+1) 612-626-7541
E-mail: york@chem.umn.edu

[b] C. S. López, O. N. Faza, Prof. A. R. de Lera
Departamento de Química Orgánica, Universidade de Vigo
Lagoas Marcosende, 36200, Vigo, Galicia (Spain)

[c] C. S. López, Prof. X. Lopez
Kimika Fakultatea, Euskal Herriko Unibertsitatea, P.K. 1072
20080 Donostia, Euskadi (Spain)
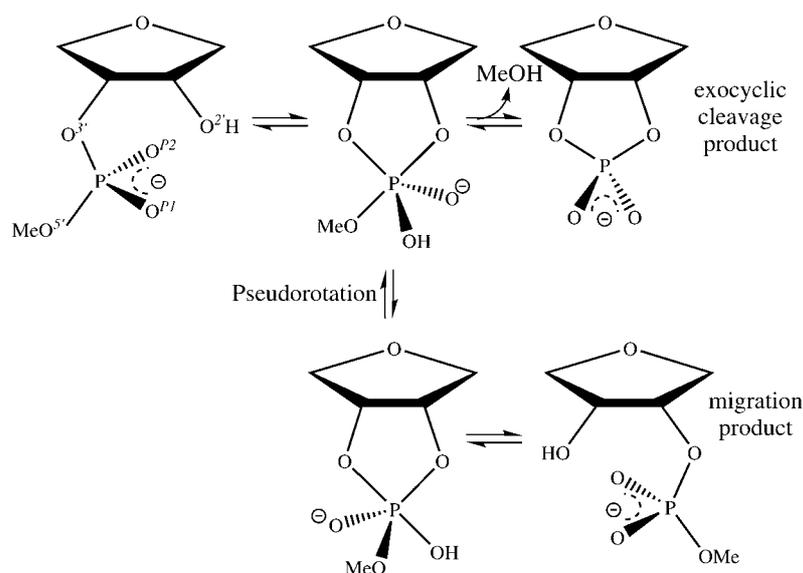
Scheme 1. Reaction pathway for transphosphorylation and migration in an RNA model system.

X: O⁻, S⁻, OH, SH (pivotal atom)
Y: OH, SH (acyclic pivoting substituent)
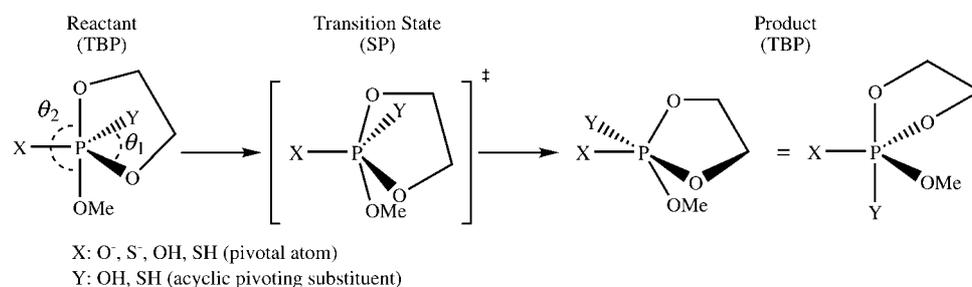
Scheme 2. Mechanism and definition of geometrical parameters for pseudorotation of cyclic oxy- and thiophosphoranes. The "reaction coordinate" q is defined as $q = (\theta_1 - \theta_2)/60$. The "ideal" reactant and product structures are trigonal bipyramidal (TBP) and have reaction coordinate values of $+1$ and -1, respectively. The ideal transition state structure is square pyramidal (SP) and has a reaction coordinate value of 0.

2. Methods

The pseudorotation barriers for model cyclic phosphoranes and thiophosphoranes with sulfur in the nonbridging oxygen positions were calculated using density functional theory (DFT) and are shown in Table 1. All the structures were optimized using the hybrid exchange functional of Becke,[14,15] the Lee, Yang, and Parr correlation functional[16] (B3LYP), and the 6–31++G(d,p) basis set, with the 6–311++G(3df,2p) basis set used for single point energy refinement.[17] Thermodynamic quantities were calculated in a manner analogous to previous work.[18] Single-point solvation calculations were performed at the optimized geometries using the polarizable continuum model (PCM)[19,20] and a variation of the conductorlike screening model (COSMO employing the parameters provided by Klamt and co-workers)[21] as implemented in Gaussian03[22] with the UAKS radii[23] and the SM5.42R solvation model[24] as implemented in MN-GSM.[25] All calculations were performed with the Gaussian03 suite of programs,[17] with the exception of the SM5 solvation calculations for which Gaussian 98 was used.

Hybrid density-functional methods (such as the B3LYP method applied in the present work) have been applied extensively to pentavalent phosphoranes and shown to be very reliable. Some recent applications include analysis of anharmonic

cyclic cleavage rates, the barrier to pseudorotation, and the lifetime of the phosphorane intermediate.[13] In the case of thio-substituted phosphates (phosphorothioates), many of the fundamental assumptions about the reactive intermediates of oxyphosphates may not be transferable.

Herein the mechanisms and barriers to pseudorotation for a series of oxyphosphorane and thiophosphorane molecules that serve as model intermediates in RNA transphosphorylation are presented. These results, together with results for in-line nucleophilic attack, provide detailed insight into biological phosphorous reactivity, and in particular, the rates and pH dependence of transphosphorylation, including the nature of thio effects, in RNA systems.

Table 1. Reaction and activation free energies in solution for pseudorotation.[a]

| Molecule | ΔGaq (298) | | | ΔG‡aq (298) | | |
	PCM	COSMO	SM5	PCM	COSMO	SM5
	neutral/diprotic					
OH, OH	2.18	2.06	2.59	6.27	6.40	6.32
OH, SH	1.02	0.99	1.30	7.72	7.65	7.71
SH, SH	2.82	2.87	2.81	6.20	6.18	5.98
	monoanionic/monoprotic					
OH, O⁻	1.71	2.00	2.37	2.64	2.75	3.00
OH, S⁻	2.23	2.10	2.41	3.32	3.37	3.43
SH, S⁻	1.32	1.24	1.28	3.44	3.27	3.02

[a] Energetic quantities are in kcal mol⁻¹. All thermodynamic quantities correspond to 298.15 K. For comparison with other ab initio results, see references [42] and [3] (and references therein).

frequencies[26] and pseudorotation[27] of PF_5, the calculation of phosphorane pK_as,[28] phosphate hydrolysis reactions,[29] and very recently, a detailed study of the structure and stability of oxyphosphoranes and related compounds.[30]

3. Discussion

Values for the rate-limiting transition state barrier for pseudorotation of the monoanionic/monoprotic species are 3–4 kcal mol^{-1} lower than the corresponding neutral/diprotic species. The gas-phase activation free energy values range from 6.26–7.83 kcal mol^{-1} for neutral phosphoranes, and 3.30–5.25 kcal mol^{-1} for monoanionic phosphoranes. The activation free energies tend to decrease in solution, but the neutral phosphoranes still have higher activation barriers compared to the monoanionic phosphoranes (5.98–7.72 and 2.64–3.44 kcal mol^{-1}, respectively). The monoanionic/monoprotic species contain a localized negative charge from the oxy or sulfide substituent in the equatorial plane. This localized negative charge unbalances the axial–equatorial repulsions and deforms the angle between the axial bonds of the trigonal bipyramid toward that of a more transition-state-like square pyramidal structure (Figure 1). The decreased energy barrier to pseudorotation for monoanionic phosphoranes is, to some extent, a result of reactant (and product) destabilization due to greater electron pair repulsions that are partially alleviated in the transition state.

The pseudorotation barriers for monoanionic and neutral oxyphosphoranes in solution are lower than those estimated from gas-phase calculations of similar species.[31] The various solvation methods give similar results for values of ΔG_{aq} and $\Delta G^{\ddagger}_{aq}$, and reveal several important trends. In general, solvation tends to lower the pseudorotation barriers for the systems studied here; a minor exception being the small barrier for the monoanionic thiophosphorane system (OH,S$^-$). For the neutral systems, the transition state solvent-stabilization is small (less than 1 kcal mol^{-1} for the cyclic oxyphosphorane), and decreases with increasing sulfur substitution. The $\Delta G^{\ddagger}_{aq}$ values for the monoanionic oxyphosphorane and monoanionic dithiophosphorane are lower than their gas-phase values by 2.25–2.61 and 0.72–1.14 kcal mol^{-1}, respectively. Solvent effects are less pronounced upon sulfur substitution since the larger radius of sulfur decreases the solute-solvent interaction.

The barriers to pseudorotation for solvated phosphoranes and thiophosphoranes in the same charge state are similar (Table 1). For the neutral phosphoranes, the largest pseudorotation barrier ($\Delta G^{\ddagger}$) is observed for the thiophosphorane (OH,SH: 7.65–7.72 kcal mol^{-1}), and the lowest barrier for the dithiophosphorane (SH,SH: 5.98–6.20 kcal mol^{-1}). The corresponding reaction free energy (ΔG) values follow the opposite trend having the largest values for the dithiophosphorane (SH,SH: 2.81–2.87 kcal mol^{-1}) and the smallest values for the thiophosphorane (OH,SH: 0.99–1.30 kcal mol^{-1}). For the monoanionic phosphoranes, the pseudorotation barriers were all quite similar, whereas the corresponding ΔG values were smallest for the dithiophosphorane and largest for the thiophosphorane.
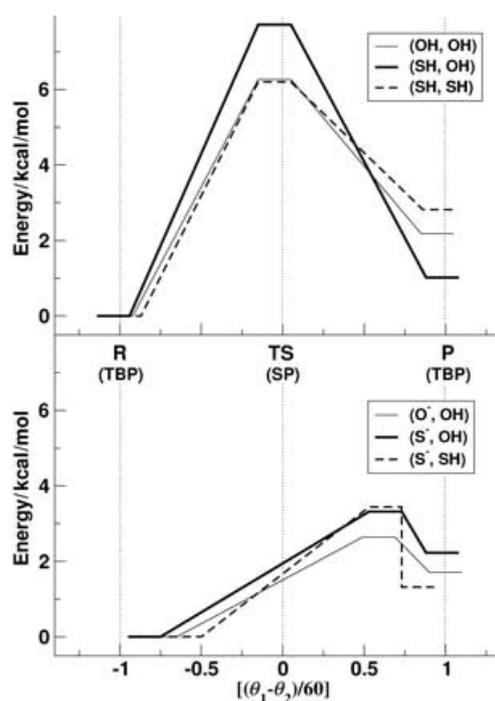
Figure 1. Reaction diagrams for pseudorotation of cyclic oxyphosphoranes and thiophosphoranes. Shown are neutral/diprotic species (top), and monoanionic/monoprotic species (bottom). The lines are labeled with the convention: (X,Y), where X indicates the pivotal atom, and Y indicates the acyclic OH or SH ligand that pseudorotates from an equitorial position in the reactant to an axial position in the product (Scheme 2). Dotted vertical lines at reaction coordinate values (q = ($\theta_1-\theta_2$)/60) of 0 and ±1 correspond to ideal SP transition state (TS) structures and TBP reactant (R) and product (P) structures, respectively. Pseudorotation for dianionic phosphoranes involves placement of an anionic ligand in the axial position. Preliminary calculations indicated this configuration to be highly unstable, as suggested by other experimental and theoretical work ,[3] and pseudorotation of the dianionic phosphoranes was not further considered.

The interplay between pseudorotation, nucleophilic attack, and ring opening in unsubstituted phosphates/phosphoranes has been studied with experimental[32–34] and theoretical methods,[35, 31] in addition to a few studies that include thio substitutions.[36] In the case of the hydrolysis of small phosphates such as methylethylenephosphate (MEP), the rate of pseudorotation and the ratio of endo- and exocyclic cleavage products are highly pH dependent.[37] Low pH values favor products that arise from pseudorotation.

The ratio of cleavage and migration products will be strongly influenced by the propensity for the reactive intermediate to undergo pseudorotation (Scheme 2). The degree of pseudorotation will depend both on the lifetime of the reactive intermediate and the pseudorotation barrier. A key aspect of the kinetics resides in the lifetime of the phosphorane intermediate which is strongly dependent on its protonation state.[2, 3]

Table 2 compares the relative free energies and transition state structures of unsubstituted model reactions calculated using DFT[38] and sulfur-substituted reactions calculated with hybrid quantum mechanical/molecular mechanical (QM/MM) methods.[39] The unsubstituted dianionic species undergoes a single-step nucleophilic displacement reaction with no kineti-

Table 2. DFT and QM/MM results for model transphosphorylation reactions in solution.[a]

Reaction TS$_{X2'}$	Relative free energy [kcal mol^{-1}] I	Rate-limiting TS TS$_{X5'}$	r_1 [Å]	r_2 [Å]
	DFT results for unsubstituted transphosphorylation			
O$^-$, O$^-$	–	–	19.59	1.845	2.509
O$^-$, OH	22.30	19.06	33.45	1.715	2.248
OH,OH	24.93	8.44	29.96	1.686	1.931
	QM/MM results for substituted dianionic transphosphorylation			
O$^-$, O$^-$	11.8	11.6	18.0	1.81	2.39
O$^-$, S$^-$	10.6	6.9	19.9	1.77	2.56
S$^-$, S$^-$	12.1	4.8	24.2	1.76	2.62

[a] Relative free energies (kcal mol^{-1}) with respect to the reactants at 298.15 K for transphosphorylation of a 2′,3′-cyclic ribose phosphate model. Shown at the top are DFT results for the unsubstituted system in dianionic, monoanionic and neutral forms using the PCM solvation model.[20] Shown at the bottom are hybrid QM/MM results[39] for the substituted 2′,3′-cyclic ribose phosphate models in the dianionic form. Also depicted are the values for the $r_1 = $P–O$^{2'}$ and $r_2 = $P–O$^{5'}$ distances at the rate-limiting transition state.

cally significant intermediate, consistent with a multitude of experimental and theoretical data.[2,3] The transphosphorylation barrier for the dianionic oxyphosphorane reaction (19.6 kcal mol^{-1}) is considerably lower than the rate-limiting barriers for the neutral and monoanionic reactions since the activated 2′-oxyanion is more nucleophilic than the corresponding hydroxy group.[4]

For the monoanionic transphosphorylation, a kinetically significant intermediate is formed with forward and reverse barriers of 14.39 and 3.24 kcal mol^{-1}, corresponding to exo- and endocyclic cleavage, respectively. The reverse barrier from the intermediate is comparable to the barrier to pseudorotation of the monoanionic oxyphosphorane (approximately 3.4 kcal mol^{-1}). Hence, in the pH range from 4–6, pseudorotation may be at least a partially rate-limiting step in isomerization.[4] The neutral reactions exhibit a deep intermediate well with forward and reverse barriers of 16.49 and 21.52 kcal mol^{-1}, respectively. Under acidic conditions the oxyphosphorane intermediate is expected to have a sufficiently long lifetime to allow pseudorotation to occur.

The results presented here are consistent with kinetic studies of the pH dependence of acid-catalyzed cleavage and isomerization reactions. This implies that uncatalyzed pseudorotation of the protonated oxyphosphorane is not rate-limiting and that a rapid equilibrium exists between structures with O$^{2'}$ and O$^{3'}$ in the apical position.[4] Taken together, these data suggest that formation of migration products result from the greatly enhanced lifetime of the neutral oxyphosphorane intermediates[2–4] and not from a decreased barrier to pseudorotation.

Although dianionic oxyphosphoranes intermediates are not expected to be kinetically significant, it is not yet clear whether the same is true for dianionic thiophosphoranes. To determine the reaction profiles for transphosphorylation thio effects under alkaline conditions, a series of hybrid QM/MM calculations have been performed for the native reaction and for single and double sulfur substitutions in the nonbridging phosphoryl positions.[39] The native reaction and sulfur substituted reactions all proceed through two transition states, and in each case the rate-limiting step corresponds to the exocyclic cleavage (Table 2). In the case of the native oxyphosphorane, there are two transition states connected by a metastable intermediate that is kinetically insignificant (barrier of 0.2 kcal mol^{-1}). Upon substitution with a single sulfur atom, the rate-limiting barrier for inline attack increases, and the intermediate free energy well becomes more pronounced. Upon double substitution, the transition state barriers are significantly increased, and the intermediate lies in a free energy well 7.3 kcal mol^{-1} below the closest transition state barrier. The lifetime of this intermediate may be sufficient to undergo protonation by solvent[40] and subsequent pseudorotation.[2]

It is noteworthy that the relative rate-limiting barrier heights of the sulfur-substituted model reactions predicted from the hybrid QM/MM calculations suggest that alkaline transphosphorylation reaction shows a considerable thio effect. This result is in some contrast with experimental results for RNA analogs with nonbridging thio substitutions that exhibit only modest thio effects.[4,41] This difference might be in part a result of the semiempirical MNDO/d Hamiltonian model applied and/or the molecular mechanical parameters. Although this aspect of the reaction is not the direct focus of the present work, it does underscore the need for further in depth study via ab initio calculations and molecular simulation with new QM/MM models.

4. Conclusions

The results of this work offer a rationale for the observed acid-catalyzed formation of migration products in reactions of RNA and small phosphate models in solution. The most striking effect of substitutions with sulfur is the production of thiophosphoranes that have pseudorotation barriers comparable to those of the corresponding oxyphosphoranes. The thiophosphoranes have longer lifetimes with respect to in-line nucleophilic substitution and, hence, lead to different pH-dependent ratios of migration/cleavage products. The data presented in this work are important for the fundamental understanding of model reactions involved in RNA catalysis and can be used to design new-generation quantum models for simulations of enzyme and ribozyme reactions with greatly improved accuracy.

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