A Journal of the Gesellschaft Deutscher Chemiker

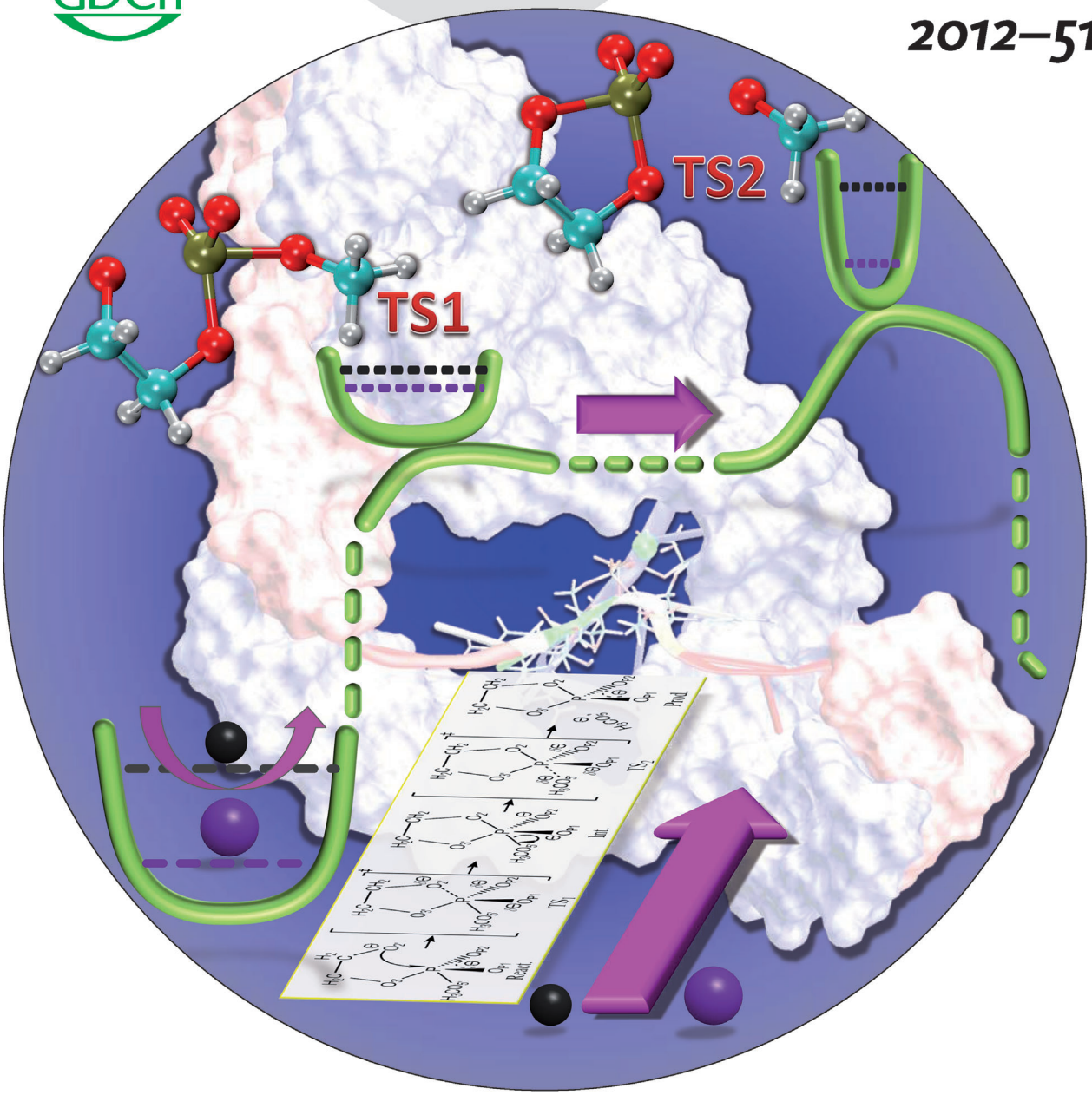
Angewandte Chemie

International Edition

GDCh

www.angewandte.org

2012–51/3



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.

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the signature of the TS1 transition state (Table 2 and Figure 2). This experimental $^{18}k_{Nu}$ 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 that were calculated for the O^{1P} and the O^{2P} atoms for the reaction with the $S^{3'}$ compound are close to unity (Table 2).

The sulfur substitution at the 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 pK_a value of primary alcohols is typically around 5 pK_a 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 $O^{2'}$ bond order). The value of Dbond in Table 1 and Figure 2 for the transition state is 0.02 Å, but this value reflects the fact that the bond length of P $S^{5'}$ is approximately 0.5 Å longer than the P $O^{5'}$ 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 5′-substituted reaction models.[13,36,37]

The primary KIE values predicted for both $^{18}k_{Nu}$ (1.042) and $^{34}k_{Lg}$ (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 ($^{18}k_{Nu}$: 1.025; $^{34}k_{Lg}$: 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 phosphoryl oxygen positions in the reaction with the native UpG dinucleotide were measured (Table 2). The present model system involves an associative reaction with a dianionic 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 phosphoryl 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 $S^{3'}$ 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.

Received: June 16, 2011
Revised: October 6, 2011
Published online: November 11, 2011

Keywords: ab initio calculations · kinetic isotope effects · reaction mechanisms · RNA · transphosphorylation

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