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Theoretical studies on the effect of sulfur substitution for the methanolysis of cyclic and acyclic phosphate esters

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ABSTRACT

The methanolysis of cyclic and acyclic phosphotriesters have been investigated at the B3LYP/6-31++G(d,p) level in the gas phase and in solution, and the effect of sulfur substitution at the attacking and leaving positions are taken into account. It is found that the activation enthalpies of the first and second steps for the methanolysis of acyclic diethyl methyl triester (DEMTE) are the same. The activation enthalpy of the second step is evidently higher than that of the first step for the thiolysis of DEMTE, while for the methanolysis of DEMTE with sulfur substitution at the leaving position (named $S_{2'}$-DEMTE), the first step is the rate-controlling step. For the methanolysis of cyclic triester (CYTE), it proceeds through an associative mechanism with the first step being the rate-controlling step, while a concerted mechanism is observed for the methanolysis reactions with sulfur substitutions. Additionally, they proceed by concerted mechanism for the methanolysis of $S_{2'}$-aryl-triesters ($S_{2'}$-ARTE), where the sulfur substitutes at the leaving position. The enthalpies of all stationary points in solution are higher than those in the gas phase, indicating the importance of the solvent effect. Our calculations provide a comprehensive data and fundamental microscopic insights into the methanolysis of cyclic and acyclic phosphotriesters.

1. Introduction

Phosphorus plays a key role in biological processes including information storage, signaling pathways and energy transfer, so the phosphoryl transfer reactions and the hydrolysis of phosphate esters have been a topic of interest for many years [1]. Among the numerous phosphates, triesters have been widely introduced into the environment as agricultural insecticides and chemical warfare nerve agents [2–4]. The researches on phosphotriesters become more and more important with the modernization and automation of agriculture. Since the experimentations of toxic nerve agents is too hazardous, computational studies are typically performed to map out the detailed reaction mechanisms for hydrolytic degradation of these toxic triesters [5].

As the intermediates in the phosphoryl transfer of ribonucleic acids RNA, the 5-membered cyclic phosphodiesters are of particular importance and have been widely studied experimentally and theoretically [6–8]. The pioneering researches have shown that the hydrolysis of 5-membered ring systems proceed much faster than the hydrolysis of their acyclic analogs [9]. Dejaegere and his co-workers calculated the energy barriers for the 5-membered cyclic and acyclic phosphodiesters in the gas phase and in solution, and the results indicated that most of the rate acceleration of the cyclic versus the acyclic phosphates arose from differential solvation of the transition states although there was strain in the ground state of the cyclic reactant [10]. Later, the C–O versus P–O bond cleavage for nucleophilic attacks in the 5-membered ring phosphate esters were performed by Ashkenazi et al. [11], and they pointed out that the C–O bond cleavage pathway should be taken into consideration for the thio effect of the phosphoryl transfer and hydrolysis of phosphate. However, up to now not much effort has been made in the direction of cyclic triesters hydrolysis, especially the comparisons of the cyclic versus the acyclic triesters.

Another question regarding the reactivity of phosphate esters is whether the nucleophilic attack process proceeds through an associative or dissociative mechanism as illustrated in Scheme 1. The associative mechanism is a limiting-case, where a trigonal bipyramidal phosphorane (TBP) intermediate is characterized after the complete bond formation to the nucleophile, and then the bond cleavage to the leaving group follows. Another limiting-case mechanism is a dissociative mechanism, it contains a metaphosphate (PO_3^-) intermediate with a complete bond cleavage to the leaving

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group and no bond formation to the nucleophile, and then PO_3^- reacts with the nucleophile. Between the two limiting-case mechanisms is a concerted mechanism, which features a bipyramidal transition state formed by synchronous bond formation and bond fission steps.

For the nonenzymatic reactions, the hydrolysis of the monoesters proceed by the dissociative mechanism containing the metaphosphate (PO_3^-) intermediate, while the associative and dissociative mechanisms for water attack on methyl phosphate are equally viable and indistinguishable in solution [12]. It has been suggested by the linear free energy relationships (LFER) [13,14] and kinetic isotope effects [15,16] that the diesters with aryl leaving groups undergo hydrolysis via a concerted pathway with a tighter transition state. For the alkaline hydrolysis reaction of 5-membered cyclic diester ethylene phosphate, an associative mechanism with a kinetically insignificant intermediate is observed [17]. The mechanism for phosphotriesters hydrolysis is thought to be associative, with a trigonal bipyramidal phosphorane (TBP) structure along the reaction pathway. While the actual character of the phosphorane structure, including whether it is a transition state or a true intermediate, and its protonation state, have been issues of considerable debate for acyclic triester over the years [18]. Plenty of studies have showed that depending on the characteristic of leaving group, it reacts by an associative mechanism for triester with a poor leaving group, while for triester with a good leaving group, it proceeds through concerted mechanism [19–23]. Though a number of proposals for the TBP phosphorane structure are found in the literature, the uncertainty of understanding the methanolysis mechanism of the cyclic and acyclic triesters warrants detailed theoretical studies as not much effort has been made in this direction.

From the early researches we know that the charge state of the phosphate [24] controls the hydrolysis and alcoholysis mechanisms. And a neutral mono or diester would involve intramolecular proton transfer which would obscure the intrinsic reactivity. So in this work we have chosen triesters though a mono- or dianion phosphate would be a more realistic representation of the PTPase enzymatic substrate [25], and we have probed the influence of the thiolate nucleophile to further explore the mechanism.

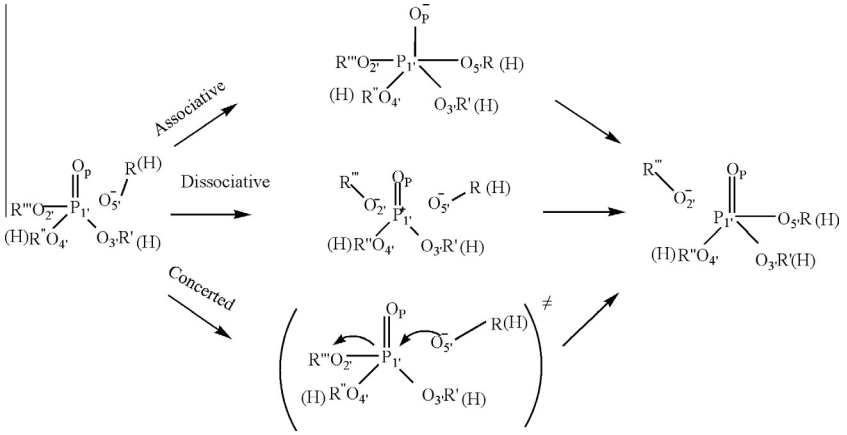
Firstly, the alcoholysis and thiolysis of phosphates are of great relevance to study the influence of the nucleophile on phosphoryl transfer and phosphate hydrolysis reactions. Secondly, both of the phosphate ester alcoholysis and thiolysis are important biochemical reactions because a large family of phosphatases catalyze the dephosphorylation of proteins and peptides through the formation of kinetically competent intermediates phosphoserine and phosphocysteine [25,26]. Moreover, the sulfur substitutions for the reactions of phosphate based on theoretical calculations offer mechanistic interpretation of kinetic data and may help to understand the intrinsic features of the structural and catalytic mechanisms of ribozymes [27,8,28,29].

Unfortunately, most nucleophilic reactions of phosphate esters are hydrolysis and alcoholysis, which have been studied in the gas phase, solution and computationally. While phosphate ester thiolysis, a biologically important reaction, has received less attention. The reaction rate constant or energy barrier change as the substitution of oxygen atom by sulfur atom is usually called "thio effect". A few previous researchers [27,8,28,30,31] have set out to analysis the sulfur substitution for the phosphate reactions, and they have showed the substitution of oxygen atom by sulfur atom, especially substituting the attacking and leaving station, would change the rate constants and mechanisms of the reactions. For example, thiols in the 2′-position of nucleotide analogs attack at the glycosidic linkage [32], the free energy barrier for the transesterification of 2′-deoxy-2′-thiouridine3′-(p-nitrophenyl phosphate) with the thiolate attack on the adjacent phosphodiester bond is 10 kcal/mol higher than that with the alkoxide [33]. The intra-molecular attack of diester 2′-hydroxypropyl-p-nitrophenyl phosphate (HPpNP) proceeds through the nucleophilic reaction with P–O bond cleavage, while for its analogous compound 2-thiouridyl-p-nitrophenyl phosphate (s-2′pNP), in which the sulfur atom substitutes the nucleophilic position, the intramolecular attack of 2′ thiouridyl group takes at the beta carbon atom characterized by C–O bond cleavage [34]. The related theoretical studies have been performed using density functional theory in our previous study [35].

In light of the many questions regarding the microscopic details of the catalyzed reactions remaining unresolved and the limited number of quantitative studies on the comparisons of the cyclic versus the acyclic triesters, in the present study, we extend and complement our previous work [36] by DFT method for the methanolysis mechanism of cyclic and acyclic triesters, aimed at an in-depth analysis of the methanolysis mechanism by exploring the thio effects. Our systemic research may constitute a reference for the analysis of the intrinsic reactivity of phosphotriesters and of the possible catalytic mechanisms of phosphatases.

2. Computational details

Geometries of the reactant complexes, transition states, intermediates and product complexes for all the reactions were fully optimized using density functional theory. The Becke three parameter exchange functional and the gradient-corrected functional of



Scheme 1. Three mechanisms for the nucleophilic reactions of phosphoryl transfer and phosphate hydrolysis.