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Comparing Spontaneous Hydrolysis Rates of Activated Models of DNA and RNA

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***Abstract*—**This research project aims to investigate difference in relative rates concerning phosphoryl transfer relevant to biological catalysis of DNA and RNA in the pH-independent reactions. Activated Models of DNA and RNA for alkyl-aryl phosphate diesters (with 4-nitrophenyl as a good leaving group) have successfully been prepared to gather kinetic parameters. Eyring plots for the pH–independent hydrolysis of **1** and **2** were established at different temperatures in the range 100–160 °C. These measurements have been used to provide a better estimate for the difference in relative rates between the reactivity of DNA and RNA cleavage. Eyring plot gave an extrapolated rate of $k_{H2O} = 1 \times 10^{-10}$ s $^{-1}$ for **1** (RNA model) and **2** (DNA model) at 25°C. Comparing the reactivity of RNA model and DNA model shows that the difference in relative rates in the pH-independent reactions is surprisingly very similar at 25°. This allows us to obtain chemical insights into how biological catalysts such as enzymes may have evolved to perform their current functions.

***Keywords*—**DNA & RNA Models, Relative Rates, Reactivity.

I. Introduction

PHOSPATE diester exists as a frequent piece to join the nucleotides in the backbone of DNA and RNA. The sugar in RNA is ribose with hydroxyl group at position C–2' (Ribonucleic acid RNA), but deoxyribose exists in DNA as a sugar (Deoxyribonucleic acid DNA), which contains a hydrogen atom at C–2' [1]-[3]. The linkage of phosphate diester in DNA is extremely stable bonds and resistant to cleave under mild conditions in aqueous solutions without efficient catalysts [4]-[6]. However, the rather simple cleavage of phosphodiester linkages in RNA in base conditions is in contrast with DNA hydrolysis. This was attributed by 2′-OH group which acts as a good intramolecular nucleophile [7]. The above mentioned nucleophile can easily cleave phosphate diesters particularly when the nucleophile deprotonated in high pH to be more effective [8]-[11]. Due to the extremely low reactivity of phosphate diesters, one approach is to use elevated temperature to estimate the corresponding reactivity at low temperature.

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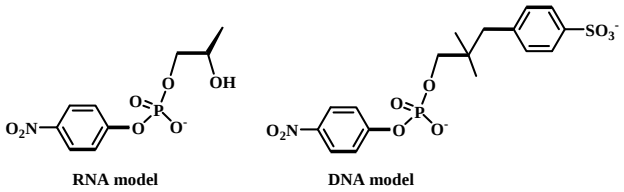

Fig. 1 Phosphodiesters used in this work, in which designed to mimic the phosphate diester linkage in DNA and RNA cleavage. 2-hydroxy-1-propyl-4-nitrophenylphosphate diester (HPNPP) 1 was used to mimic RNA phosphate cleavage and the phosphate diester backbone of DNA mimicked by 4-nitrophenyl-(2,2-dimethyl-3-(4-sodium sulfonyl phenyl) propyl) phosphate diester (4-NPNp*P) 2.

II. Results and Discussion

In an effort to determine the relative hydrolysis rates for DNA and RNA in water reactions, and to estimate the difference in the reactivity between RNA and DNA hydrolysis, we suggested to develop models for the key structure of dialkyl phosphates in DNA and RNA. Fig. 1 shows chosen substrates to mimic the phosphate diester cleavage of DNA and RNA. Two activated models for the key structure of DNA and RNA have successfully been established by replacing real linkage group of alkyl group with the p*K*a of the 3′ or 5′ OH as a leaving group ≈ 14.3 [12] in DNA and RNA by a better leaving group with pK_a ≈ 7.14 as shown in Fig. 1. Designed simple models can provide a good estimate for the difference in the reactivity between RNA and DNA hydrolysis at low temperature.

In this work, we determined the rates of spontaneous hydrolysis of (HPNPP) **1** and (4-NPNp*P) **2**, in which P–O bond cleavage occurs, but C–O bond cleavage is prevented by steric effects as shown in (Fig. 1). To estimate the difference in reactivity between DNA and RNA hydrolysis, we measured the relative rates of hydrolysis of **1, 2** at different temperatures in pH-independent, using 4-nitrophenyl as a leaving group for the cleavage of DNA and RNA. Fig. 2 shows established pH-rate profile for the pH-independent hydrolysis of 4-NPNp*P (**2**) at 100°C and HPNPP (**1**) at 37°C over a range of pH (measured at 100°C), along with a published pH-rate profile for RNA at 90°C [13], [14]. Studied models exhibit acid and base catalysis regions with different ranges for the pH-independent region. For example, 4-NPNp*P (**2**) shows the same rate constant from pH 4 to 10, while RNA model exhibits a pH minimum around pH 4.7 with continuous curvature without complete flattening. Thus, spontaneous hydrolysis is only observed clearly around pH = 4.7 in UpU

model (13-14) and over a wide range for DNA model from pH 4 to 10.

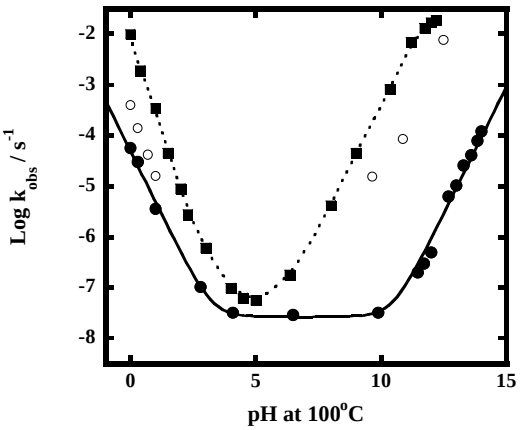

Fig. 2 pH-rate profile for the pH-independent hydrolysis of (■) is for (UpU) at 90°C and (●) is 4-NPNp*P (2) at 100 °C over a range of pH (measured at 100°C). (○) is HPNPP (1) at 37°C

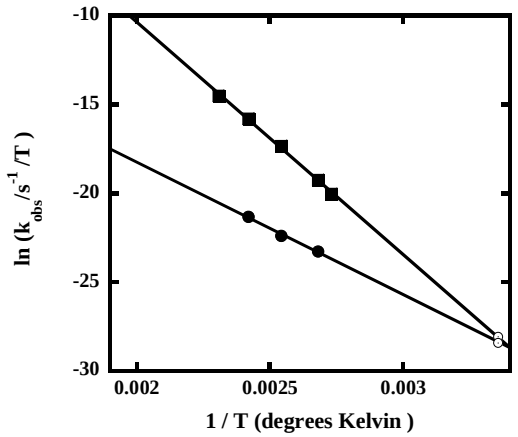

Fig. 3 Relationship between different temperatures (100 – 160°C) and rate constants (s^{-1}) (■) represents pH–independent hydrolysis of HPNPP (1) in 0.03 M acetate buffer (pH 4.7) and (●) is spontaneous hydrolysis of (4-NPNp*P) 2 in 0.05 M potassium phosphate buffer (pH 6.5)

Fig. 3 shows an Eyring plot of good results obtained in 0.05 M potassium phosphate buffer pH 6.5 for **2** and 0.03 M potassium acetate buffer pH 4.7. Extrapolating of obtained results indicates that, at 25°C, the apparent first-order rate constant is $k_{25} = 1 \times 10^{-10}$ s^{-1} for hydrolysis of **1** and **2**. An Eyring plot based on data points gathered over the lowest reactivity of pH according to pH-rate profiles of **1** and **2**, indicates that $\Delta H^{\ddagger}$ 62.2 kJ mol^{-1} for DNA model reaction, and $\Delta S^{\ddagger}$ –165 J mol^{-1} K^{-1}, but RNA model gives $\Delta H^{\ddagger}$ 108.9 kJ mol^{-1} and $\Delta S^{\ddagger}$ –65.9 J mol^{-1} K^{-1}. The activation parameters for the hydrolysis of 4-NPNp*P (2) is very similar to that for Me-2,4-DNPP- and *bis*-2,4-DNPP- in H_2O, $\Delta H^{\ddagger}$ 59.5 and 79.5 kJ mol^{-1}, $\Delta S^{\ddagger}$ –131 and -107 J mol^{-1} K^{-1} [15]-[16].

The similarity between the extrapolated rates of DNA and RNA models at 25°C, implies that under neutral conditions seems the hydroxyl group at position C–2' does not play a clear role in the mechanism. Thus, it seems reasonable to draw a mechanism as shown below, where the substrate acting as a general base is a more likely mechanism than the direct attack of H_2O as a nucleophile.

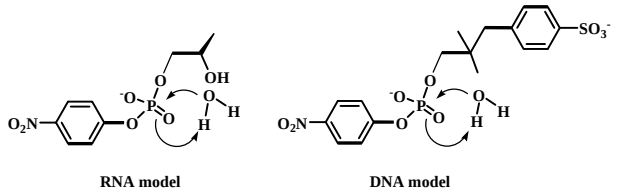

Fig. 4 Suggested mechanism for the cleavage of RNA and DNA models (**1** and **2**) in pH–independent reactions.

Measurements of the hydrolysis of 1 and 2 as simple alkyl-aryl phosphate diesters have been used to provide a better estimate of the stability of DNA and RNA towards hydrolytic P–O cleavage, ($k_{25} = 1 \times 10^{-10}$ s^{-1} at 25°C for both).

III. Conclusion

The kinetic results presented here provide strong evidence of the similar mechanism for the cleavage of DNA and RNA under pH-independent conditions. Suggested mechanism can be drawn according similar extrapolated rates for activated models of DNA and RNA, which seems there is no an important role for the hydroxyl group at position C–2' (Ribonucleic acid RNA) as expected for that big role in basic reactions (Fig. 4).

IV. Materials and Methods

2-hydroxy-1-propyl-4-nitrophenylphosphate diester **1** was prepared according to published procedure (17). 4-nitrophenyl-alkyl phosphate diester was successfully prepared by reacting 1 eq. of 2,2-dimethyl-3-phenyl propanol with a solution of 1 eq. of 4-nitrophenyl phosphoro-dichloridate with TEA in dry DCM while stirring at 3°C. The reaction mixture was left to reach room temperature and then stirred overnight under a nitrogen atmosphere. A solution of water with TEA was added into the reaction mixture to form the phosphate diester triethylammonium salt. Removal of the solvents yields the crude product with some by products. Consequently, the crude product was purified by silica gel chromatography and sulfonated to give 4- nitrophenyl-2,2-dimethyl-3-phenyl propanyl phosphate sodium salt **2**.

Kinetic experiments were carried out in the designed stainless steel vessels at 100 - 160 ± 0.1°C in a circulating oil bath. Calculated amount of diester was dissolved in buffer solution containing 2.5 mg of benzoic acid as an internal reference. compound **1** (19.6 mg, 0.044 mmol) was dissolved in pre-heated solutions of phosphate or acetate buffers at needed temperature to yield a solution of 20 mM of the diester containing 4mM of the internal reference. The reaction mixture was loaded into the reaction vessels after tightening using a torque wrench and fully placed in a Grant circulating oil bath, the temperature of the bath was monitored and

maintained using a digital microprocessor thermometer. Aliquots from the reaction vessels (50 µl) were taken at the desired time intervals after cooling. The sample was filtered by Chromacol 0.2 µm syringe filter and analyzed by HPLC at λ_{max} 224 nm, where all materials (substrate and products) can be monitored, benzoic acid selected as stable internal reference containing a similar chromophore at 224 nm. The 40 µl was injected to completely fill the loading loop (20 µl). This was loaded onto the column and eluted with solvent system of Acetonitrile and 0.1% TFA in H_2O at a flow rate of 1 ml min-1. The pseudo-first-order rate constant was obtained by plotting the relative peak area against time.

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