Imidazole-Mediated Phosphodiester Hydrolysis.Analysis of Phospholipase D Mechanism Through the Eyes of Mimic Models

Joel I. Santos (IC)[1], Mozart S. Pereira (PG), Tiago A. S. Brandão (PQ)*

Departamento de Química, ICEx, Universidade Federal de Minas Gerais, 31270-901, Belo Horizonte/MG
*E-mail: joel.ingracia@gmail.com[1]; tasb@ufmg.br**

Keywords: Intramolecular catalysis, Phosphoimidazole, Phosphoryl transfer, Phospholipase D.

Abstract

The P-O bond cleavage mechanism of *ortho*-(2'-imidazolyl)naphthyl 4-aryl phosphates was explored.

Introduction

Cleavage of phosphate diesters under non catalytic conditions may take million years.[1] However, 10^{12}-fold faster reactions are often achieved in the presence of enzymes.[2] Phospholipase D (PLD) catalyzes the phosphate diesters cleavage bynucleophilic attack of an imidazole group on the phosphorus atom of the substrate. Then, thephosphoimidazole formed reacts to products with nucleophilic attack of an external nucleophile.[3]This work aims the study of the imidazole-mediated catalysis in *ortho*-(2'-imidazolyl)naphthyl aryl phosphates with a close look into the features of the transition state at the first catalytic step.

Results and Discussion

Reaction rates for cleavage of 3-(2'-imidazolyl)-2-naphthyl arylphosphates, *i.e.* 4-Cl for 3Ni2P-pCl and 4-NO$_2$ for 3Ni2P-pNP, wereobtained in different pH values (Fig. 1) using UV-Vis spectroscopy.Reaction species were determined by NMR and ESI-MS techniques using reported procedures.[4]
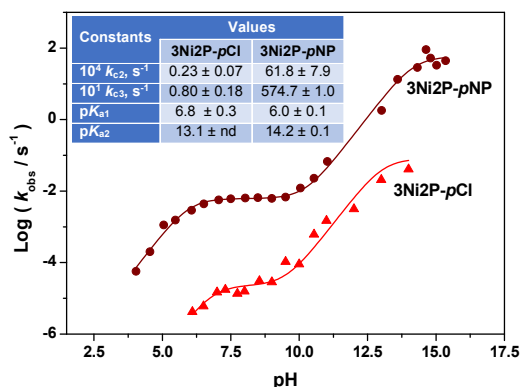
Constants	Values	
	3Ni2P-*pCl*	**3Ni2P-*pNP***
10^4 k_{c2}, s^{-1}	0.23 ± 0.07	61.8 ± 7.9
10^1 k_{c3}, s^{-1}	0.80 ± 0.18	574.7 ± 1.0
pK_{a1}	6.8 ± 0.3	6.0 ± 0.1
pK_{a2}	13.1 ± nd	14.2 ± 0.1

Fig. 1.pH-rate profiles for cleavages of 3Ni2P-pCl and 3Ni2P-pNP in water at 45.0 ºC; 0.01 M Buffer and Ionic strength 1.0 (KCl). Values in the insert are parameters to obtain the fits according to the equation:

$$\log k_{obs} = \log (k_{c2}.\chi_{3Ni2P\text{-}pNP^-} + k_{c3}.\chi_{3Ni2P\text{-}pNP^{2-}})$$

Reaction rates depend on the molar fractions of the ionic species as shown in Scheme 1. Cleavage of compounds is faster in basic conditions as the imidazolium is deprotonated to afford the nucleophilic imidazole and imidazolate.

Scheme 1. Reaction pathway for cleavage of 3-(2'-imidazolyl)-2-naphthyl aryl phosphates for different ionic species as the pH increases.
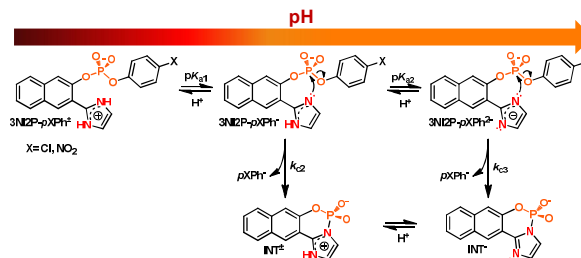
Differences in the rate constants for 3Ni2P-pCl and 3Ni2P-pNP reflect the higher electron withdrawing capacity of the NO$_2$ in relation to the Cl group. A Hammett plot for the k_{c2} parameter gives a ρ of +4.7, which is indicative of a late transition state showinghigh negative charge at the leaving group.

This observation agrees quite well with heavy isotope effect studies using the competitive methodat room temperature and pH 9.0 (50 mM Tris) for cleavage of^{15}NO$_2$ or ^{14}NO$_2$-substituted 1-(2'-imidazolyl)-2-naphthyl 4-nitrophenylphosphates. This ^{15}N KIE was 1.0021±0.0002, which is about 70% of the maximum value of ~1.003 for transition states with extensive P-O bond fission with the leaving group*p*-nitrophenolate.[5]

B3LYP/6-31+G(d,p)/PCM (solv. water) computations give a synchronous transition state for the attack of the imidazole's nitrogen atom and the departure of the leaving group's oxygen atom at respective 2.010 and 2.044 Å of the phosphorus atom. The free energy computed was 22.6 kcal.mol^{-1} at 25 ºC, in agreement to the experimental value of 22.3 kcal.mol^{-1}.

Conclusions

The cleavage of*ortho*-(2'-imidazolyl)naphthyl aryl phosphates occurs through near synchronous TS with the leaving group departure without charge neutralization.

Thanks

[1]Lad, C. *et al.Proc. Nat. Acad. Sci. U.S.A.*, v. 100, p. 5607, **2003**.
[2]Cleland, W. W.; Hengge, A. C. *Chem. Rev.*, v. 106, p. 3252, **2006**.
[3]Selvy, P.E. *et al.Chem. Rev.*, v. 111, p. 6064, **2011**.
[4]Orth, E.S. *et al.J. Am. Chem. Soc.*, v. 132, p. 8513, **2010**.
[5]Hengge, A. C. *Acc. Chem. Res.*, v.35, p. 105, **2002**.