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Unique Catalytic Mechanism of Phosphatidylinositol-Specific Phospholipase C from *Streptomyces antibioticus*

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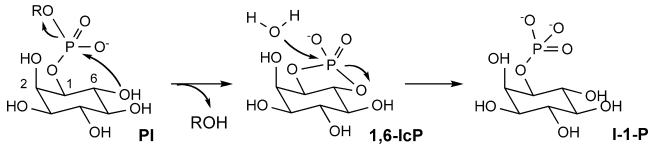
Calcium-dependent phosphatidylinositol-specific phospholipase C from *Streptomyces antibioticus* (saPLC1)[1] employs a unique catalytic mechanism whereby the cleavage of phosphatidylinositol (PI) into inositol 1-phosphate (I-1-P) occurs via a trans cyclization mechanism involving formation of inositol 1,6-cyclic phosphate (1,6-IcP; Scheme 1) as an intermediate.[2] In all other previously studied phospholipases C, this process is known to occur by cis cyclization to form inositol 1,2-cyclic phosphate as the intermediate.[3]

Scheme 1. Mechanism of saPLC1-Catalyzed Hydrolysis of PI



In this work, we examined the kinetics and products of saPLC1 reactions with phosphorothioate and phosphorodithioate analogues of PI.[4] To avoid complications related to interfacial interactions between aggregated substrate and saPLC1, we synthesized short-chain (dihexanoyl) analogues of PI (Figure 1), which exist as monomers at the concentrations used for the kinetic measurements.[5]

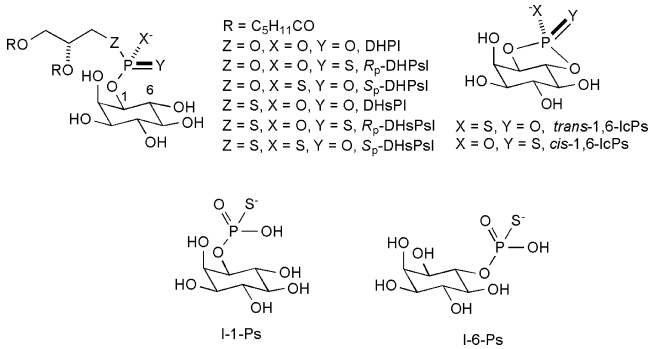


Figure 1. Structures of the PI, IcP, and IP analogues used in this work.

In contrast to the reaction with the natural PI substrate, where the cyclic intermediate is not observable, cleavage of (R_p+S_p)-DHPsI (Figure 1) proceeded with the release of the cyclic phosphorothioate (Figure 2, $\delta_{^{31}P}$ = 72.4 ppm). The intermediate was generated only from R_p-DHPsI and was identified as *myo*-inositol *cis*(2-OH,S)-1,6-cyclic phosphorothioate (*cis*-1,6-IcPs), which had been previously obtained by chemical cyclization of S_p-4-nitrophenyl inositol phosphorothioate.[6] The cyclic intermediate was gradually hydrolyzed to inositol 1-phosphorothioate (I-1-Ps; $\delta_{^{31}P}$ = 44.1 ppm); the identity of the latter was established by comparison of its ^{1}H NMR spectrum with that of genuine I-1-Ps generated by *Bacillus* PI-PLC. In agreement with our previous observations,[4] the cleavage of S_p-DHPsI was extremely slow (Table 1). In contrast, both the R_p and S_p isomers of the *phosphorodithioate* analogue, DHsPsI, were readily cleaved, with the S_p isomer being hydrolyzed only ~6 times more slowly than the R_p isomer (Figure 2 and Table 1). The low R_p/S_p stereoselectivity of saPLC1 in this case is due to a very large increase (4.8×10^6-fold) in the rate of cleavage of the S_p isomer upon introduction of the bridging sulfur (S_p-DHPsI → S_p-DHsPsI). On the other hand, the rates of cleavage of two other substrates in which the *pro-S* oxygen is left unaltered, R_p-DHPsI and DHPI, were affected to only a small extent (0.6 and 1.6-fold, respectively; Table 1) by the analogous modification of the bridging position.

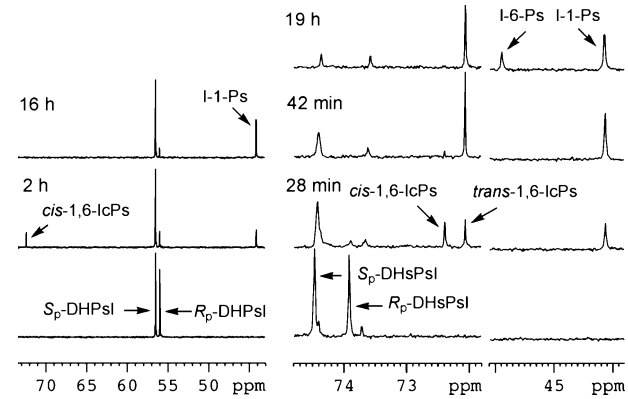


Figure 2. ^{31}P NMR time courses of the cleavage of (A) (R_p+S_p)-DHPsI and (B) (R_p+S_p)-DHsPsI by saPLC1.

Table 1. Kinetic Parameters for the Cleavage of PI and its Analogues by saPLC1

substrate	V_{max} (μmol mg^{-1} min^{-1})	k_O/k_S (nonbridging)	k_O/k_S (bridging)
DHPI	46.7 ± 1.4		
DHsPI	28.7 ± 3.9		1.6 ± 0.27
R_p-DHPsI	1.21 ± 0.07	38.6 ± 3.5	
R_p-DHsPsI	1.87 ± 0.08	15.3 ± 2.7	0.65 ± 0.06
S_p-DHPsI	≤1.41 × 10^{-6}	≥**3.0** × **10^8**	
S_p-DHsPsI	0.29 ± 0.02	99 ± 20	≥**4.8** × **10^6**

In addition to the dramatic increase in the rate of the cleavage of S_p-DHsPsI, this reaction also afforded a product ($\delta_{^{31}P}$ = 45.9 ppm) distinct from I-1-Ps. ^{1}H NMR analysis of the isolated product suggested that it was inositol 6-phosphorothioate. This conclusion was confirmed by the synthesis of I-6-Ps from *myo*-inositol and comparison of its ^{1}H and ^{31}P NMR data with those of the enzyme product.

Our results provide the following new insights into the catalytic mechanism of saPLC1: (A) The release of the 1,6-IcPs intermediate

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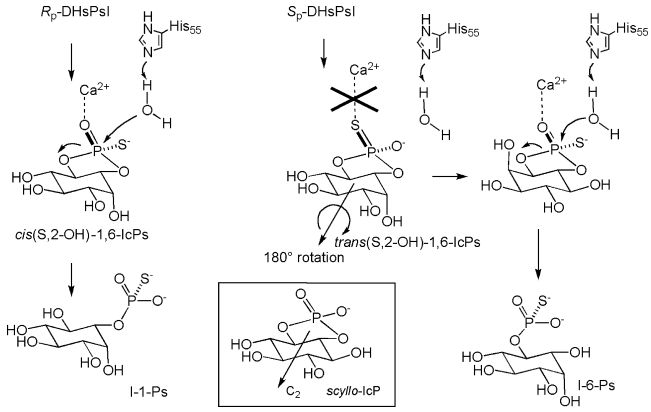
during cleavage of R_p-DHPsI is reminiscent of that in the reaction of the H16A mutant of saPLC1 with PI substrate, where formation of 1,6-IcP was detected.[2] The two results collectively suggest that the interaction of His16 with the *pro-R* oxygen atom is important for the retention of 1,6-IcP in the active site (Scheme 2). Mutation

Scheme 2. Explanation of the Formation of I-6-Ps from S_p-DHsPsI



of His16 or introduction of sulfur at the *pro-R* position abolishes this interaction, allowing the cyclic phosphate to escape from the active site. (B) The very large S_p-nonbridging thio effect[7] ($\geq 3.0 \times 10^8$) is comparable to that observed previously (1.6×10^8).[4] However, it was not clear previously whether this effect arises from a steric clash of the active site with a bulky sulfur atom in the transition state[8] or a loss of critical catalytic interactions upon the O → S modification. The fact that the sterically larger S_p-DHsPsI has a higher turnover rate than S_p-DHPsI indicates that the strong S_p thio effect arises from a loss of a catalytic interaction rather than a steric effect. (C) The very strong inverse bridging thio effect and the fact that it is observed only for S_p-DHsPsI are intriguing. In general, the magnitude of the bridging thio effect is determined by the balance of two factors: (i) the loss of general acid catalysis due to the poor proton acceptor ability of a mercaptide leaving group (Lg) and (ii) the greater stability of mercaptide versus alkoxide Lg's. A relatively modest inverse effect (1.9×10^{-3}) was previously observed for the H55A mutant of saPLC1.[4] The much greater effect observed here for the wild-type enzyme is consistent with a change of mechanism in which the cleavage of S_p-DHsPsI proceeds via a transition state with a higher negative charge on the Lg. This shift to a more dissociative mechanism likely arises from the lack of stabilization of the negative charge on the nonbridging atom. (D) Even more interesting and unexpected is the formation of I-1-Ps and I-6-Ps by hydrolyses of *cis*- and *trans*-1,6-IcPs, respectively. For hydrolysis of the cyclic phosphate, replacement of the trans oxygen of the phosphodiester by sulfur should bring about a very large detriment to catalysis, similar to that for S_p-DHPsI. Thus, a very large (10^5) nonbridging thio effect was observed for the hydrolysis of *cis*-1,2-IcPs by *Bacillus* PLC.[9] However, saPLC1 hydrolyzes *trans*-1,6-IcPs only 1 order of magnitude more slowly than *cis*-1,6-IcPs. To explain this, it is necessary to note that 1,6-IcPs is structurally similar to C_2-symmetric *scyllo*-inositol cyclic phosphate (*scyllo*-IcP), in which the nonbridging oxygens are homotopic (Scheme 2). Thus, *trans*-1,6-IcPs could bind in a rotated orientation in which the position of its nonbridging oxygen atom is the same as that in the cis isomer. The consequence of this rotated binding mode is the change of the Lg from 6-OH to 1-OH, resulting in the formation of I-6-Ps.

In summary, saPLC1 constitutes a unique enzyme that shows an unprecedented strength of interactions, as manifested by the highest bridging and nonbridging thio effects ever reported. While the role of saPLC1 in *Streptomyces* is unknown, this enzyme is capable of cleaving a number of structural analogues of PI, such as *scyllo*- and L-*chiro*-PI, as well as 2-*O*-glycosylated PIs (Bai, C., Bruzik, K. S., unpublished work). The latter have been identified as constituents of cell membranes in bacteria, protozoa, fungi, and plants.[10] Investigation of the range of structures of saPLC1 natural substrates is underway.

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Supporting Information Available: Synthesis of DHsPsI and I-6-Ps; details of the enzymatic reactions presented in Table 1 and Figure 2. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Iwasaki, Y.; Tsubouchi, Y.; Ichihashi, A.; Nakano, H.; Kobayashi, T.; Ikezawa, H.; Yamane, T. *Biochim. Biophys. Acta* **1998**, *1391*, 52.
(2) Bai, C.; Zhao, L.; Rebecchi, M.; Tsai, M.-D.; Bruzik, K. S. *J. Am. Chem. Soc.* **2009**, *131*, 8362.
(3) Heinz, D. W.; Essen, L. O.; Williams, R. L. *J. Mol. Biol.* **1998**, *275*, 635.
(4) Zhao, L.; Liu, Y.; Bruzik, K. S.; Tsai, M.-D. *J. Am. Chem. Soc.* **2003**, *125*, 22.
(5) Liu, Y.; Mihai, C.; Kubiak, R. J.; Rebecchi, M.; Bruzik, K. S. *ChemBioChem* **2007**, *8*, 1430.
(6) Kubiak, R. J.; Bruzik, K. S. *J. Am. Chem. Soc.* **2001**, *123*, 1760.
(7) Stivers, J. T.; Nagarajan, R. *Chem. Rev.* **2006**, *106*, 3443.
(8) Zhang, Y.-L.; Hollfelder, F.; Gordon, S. J.; Chen, L.; Keng, Y.-F.; Wu, L.; Herschlag, D.; Zhang, Z.-Y. *Biochemistry* **1999**, *38*, 12111.
(9) Hondal, R. J.; Zhao, Z.; Riddle, S. R.; Kravchuk, A. V.; Liao, H.; Bruzik, K. S.; Tsai, M.-D. *J. Am. Chem. Soc.* **1997**, *119*, 9933.
(10) Suzuki, E.; Tanaka, A. K.; Toledo, M. S.; Levery, S. B.; Straus, A. H.; Takahashi, H. K. *Biochim. Biophys. Acta* **2008**, *1780*, 362.

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