

Trans-Cyclization of Phosphatidylinositol Catalyzed by Phospholipase C from *Streptomyces antibioticus*

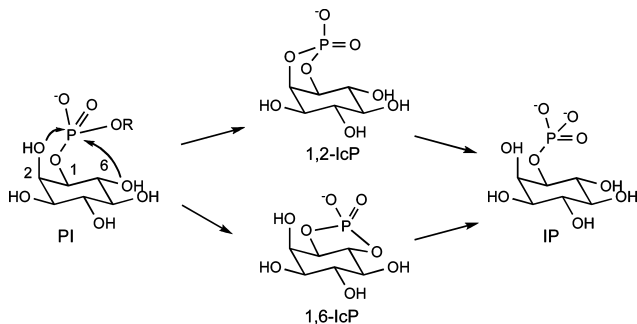
Chuan Bai,[†] Li Zhao,[‡] Mario Rebecchi,[§] Ming-Daw Tsai,^{‡,||} and Karol S. Bruzik^{*†}

Department of Medicinal Chemistry and Pharmacognosy, The University of Illinois at Chicago, Chicago, Illinois 60612, Department of Chemistry, The Ohio State University, Columbus, Ohio 43210, Departments of Anesthesiology and Physiology and Biophysics, School of Medicine, State University of New York, Stony Brook, New York 11794, and Institute of Biological Chemistry, Academia Sinica, Taipei 115, Taiwan

Received March 28, 2009; E-mail: kbruzik@uic.edu

Phosphatidylinositol-specific phospholipases C (PI-PLCs) catalyze the hydrolysis of phosphatidylinositols (PI) to inositol phosphates (IP) and diacyl glycerol, which is one of the most important steps in transmembrane signaling pathways. The catalytic mechanisms of the bacterial Ca^{2+} -independent and mammalian Ca^{2+} -dependent PI-PLC have been thoroughly investigated.^{1–3} The results of the studies performed so far indicate that both PI-PLCs catalyze the conversion of PI to IP in two chemical steps, with formation of 1,2-cyclic *myo*-inositol phosphate (1,2-IcP) as an intermediate (Scheme 1).

Scheme 1. Alternative Mechanisms of Phosphatidylinositol Cleavage by PI-PLC Involving Formation of Inositol 1,2- and 1,6-Cyclic Phosphates As Intermediates



Recently, we have embarked on a study of the Ca^{2+} -dependent PI-PLC from *Streptomyces antibioticus* (saPLC1)⁴ that we considered a simpler and more readily available model to study the mechanism of complex mammalian isozymes.⁵ Because of its homology with PI-PLC δ_1 , we initially assumed the analogous mechanism for this enzyme.⁵ We now report that saPLC1 catalyzes the hydrolysis of PI via a different mechanism involving formation of an unusual *trans*-1,6-cyclic *myo*-inositol phosphate (1,6-IcP), rather than the common *cis*-1,2-IcP.

While the wild-type saPLC1 does not release the cyclic intermediate at any detectable concentration, the reaction catalyzed by the H16A mutant clearly shows formation of the cyclic phosphate (Figure 1) giving rise to a ³¹P NMR signal at 18.1 ppm, followed by its hydrolysis to inositol 1-phosphate at 4.5 ppm, as the end product. The cyclic phosphate produced in this reaction is different from 1,2-IcP formed by the earlier investigated PI-PLCs, as shown by the addition of the standard IcP (Figure 1C). The presence of the large H-2 coupling to phosphorus (³J_{H-³¹P} = 20

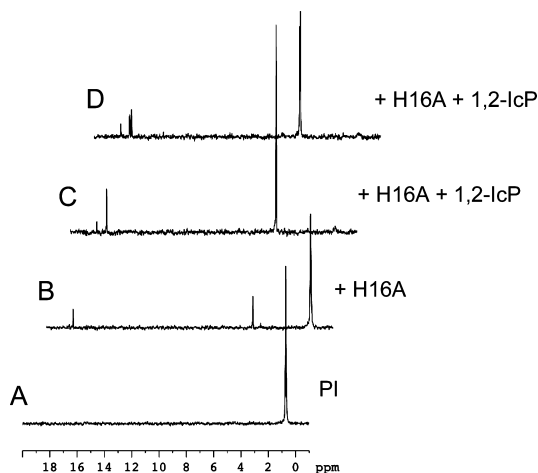


Figure 1. ³¹P NMR spectra of the reaction mixture of phosphatidylinositol treated with H16A mutant of saPLC1. (A) Phosphatidylinositol dispersed in sodium deoxycholate detergent; (B) same as A after adding H16A mutant of saPLC1; (C) reaction mixture after adding *cis*-1,2-IcP showing the enzymatically formed 1,6-IcP at 18.1 ppm and 1,2-IcP at 17.1 ppm; (D) same as C, except the spectrum was obtained with ¹H coupling.

Hz) is indicative of the *cis*-geometry of the five-membered ring in 1,2-IcP (Figure 1D). The lack of such a large ¹H–³¹P coupling (<2 Hz) together with the characteristic chemical shift indicates that the cyclic intermediate produced by saPLC1 is 1,6-IcP (Scheme 1) that we obtained earlier by chemical cyclization of inositol phosphoesters.⁶

To further characterize this intermediate we have prepared it by a combined chemical and biochemical approach. The treatment of *myo*-inositol *S*-benzyl phosphorothiolate with ammonium hydroxide produced a 3:1 mixture of 1,2- and 1,6-IcP.⁶ The mixture was treated with *B. thuringiensis* PI-PLC (btPLC) that resulted in the selective conversion of the cyclic phosphate at a higher field (*cis*-1,2-IcP) to inositol 1-phosphate, as expected (Figure 2A, middle). The remaining 1,6-cyclic phosphate was isolated and characterized by ¹H, ³¹P NMR and MS (see Supporting Information). Similarly, the treatment of the mixture of *myo*-inositol 1,2- and 1,6-cyclic phosphate 4-phosphates with mammalian PLC δ_1 resulted in the selective hydrolysis of 1,2-cyclic phosphate (Figure 2B). These experiments clearly confirm that neither of the two major types of enzymes processes the 1,6-cyclic phosphate. In contrast, the treatment of (1,2 + 1,6)-IcP with saPLC1 resulted in the disappearance of the lower field signal to produce inositol 1-phosphate as a final product. We can estimate that the rate of the cleavage of 1,6-IcP by saPLC1 is at least 10³-times higher than that of 1,2-IcP.

The formation of a five-membered cyclic transition state with *cis*-arrangement of the attacking OH nucleophile and the phosphoryl

[†] The University of Illinois at Chicago.

[‡] The Ohio State University.

[§] State University of New York.

^{||} Academia Sinica.

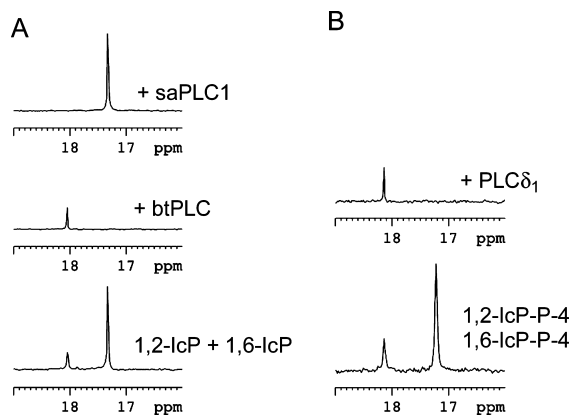


Figure 2. Specificity of hydrolysis of a mixture of inositol 1,2- and 1,6-cyclic phosphates with various PI-PLC species as monitored by ^{31}P NMR. (A) Starting mixture of 1,2- and 1,6-cyclic phosphates (bottom); after adding btPLC (middle); after adding saPLC1 (top). (B) Starting mixture of inositol 1,2- and 1,6-cyclic phosphate 4-phosphate (bottom); after adding PLC δ_1 (top).

group has been a characteristic feature of phosphoryl transferases such as RNase A and PI-PLC. For RNase, this mechanism is favored by the flexibility of the ribose ring system and close juxtaposition of the attacking 2'-hydroxy group. As we have shown earlier,⁶ however, the *cis*-cyclization of inositol phosphodiester is several hundred times slower than that of ribose phosphodiester, and there is only a minor advantage for the *cis*- vs *trans*-cyclization. Notwithstanding, all the previously investigated PI-PLC species adopted the same mechanistic paradigm as that of RNase A. Our study demonstrates for the first time that a PI-PLC-catalyzed P–O ester bond cleavage reaction can proceed through an alternative,

albeit somewhat less favorable, *trans*-cyclization process to form a 1,6-cyclic intermediate. It is quite possible that the natural target of saPLC1 could be a different isomer of an inositol phospholipid, such as *scyllo*-PI, for which only *trans*-cyclization is possible. The presence of *scyllo*-PI has been documented in both bacterial and plant systems,^{7–9} and *scyllo*-inositol itself is abundant in mammalian systems and other vertebrates,¹⁰ but to date no enzyme capable of cleaving *scyllo*-PI has been discovered.

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Supporting Information Available: Chemoenzymatic synthesis of 1,6-cyclic inositol phosphate and the corresponding ^1H , ^{31}P NMR and MS data; experimental conditions for enzymatic reactions represented in Figures 1 and 2. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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