Phosphorus, Sulfur, and Silicon and the Related Elements

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gpss20

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Published online: 06 Aug 2008.

To cite this article: Pei-Jung Lu & Ching-Shih Chen (1996) Modulation of the Inositol 1,4,5-Trisphosphate Receptor by Inositol Phosphates, Phosphorus, Sulfur, and Silicon and the Related Elements, 109:1-4, 325-328

To link to this article: http://dx.doi.org/10.1080/10426509608545156

Phosphorus, Sulfur, and Silicon, 1996, Vols. 109–110, pp. 325–328
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MODULATION OF THE INOSITOL 1,4,5-TRISPHOSPHATE RECEPTOR BY INOSITOL PHOSPHATES

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Abstract The mode of recognition at the binding site of the Ins(1,4,5)P_3 receptor was assessed by examining the structure-activity relationships of different Ca^{2+}-mobilizing inositol phosphates.

Key Words: the Ins(1,4,5)P_3 receptor; inositol phosphates; calcium mobilization; rat brain microsomes; calcium signaling

INTRODUCTION

The pivotal role of D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] in intracellular Ca^{2+} signaling is well recognized. In the cytosol, Ins(1,4,5)P_3 and its metabolites undergo extensive metabolism by the sequential actions of specific phosphatases and kinases, from which a plethora of inositol phosphates are produced (Figure 1).

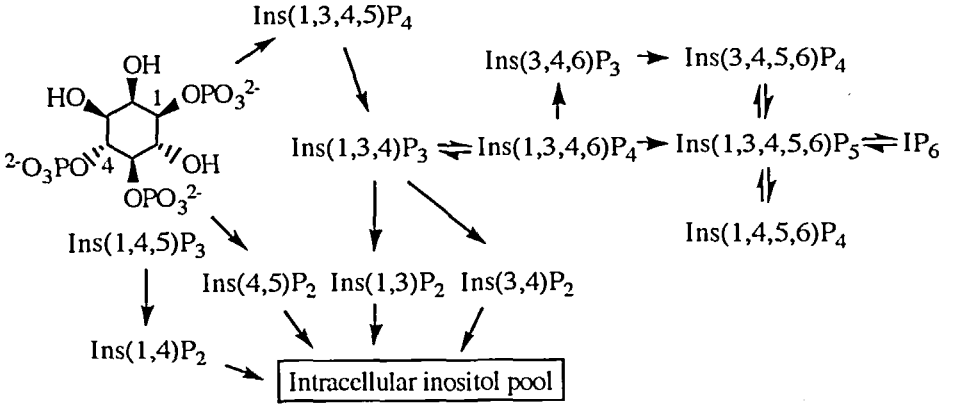


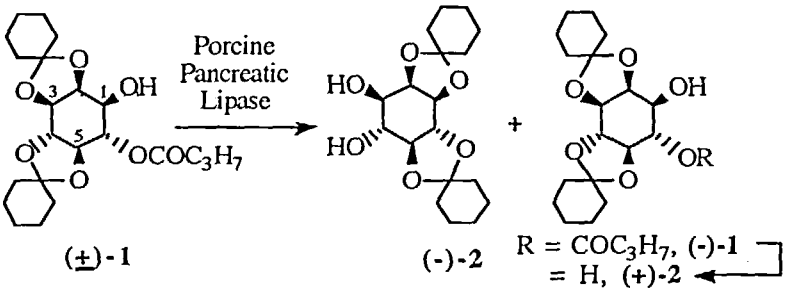
Figure 1 Metabolism of inositol phosphates

The rich diversity of phosphoinositols generated from such a complex metabolic network implies the physiological relevance of these molecules. Thus, examination of

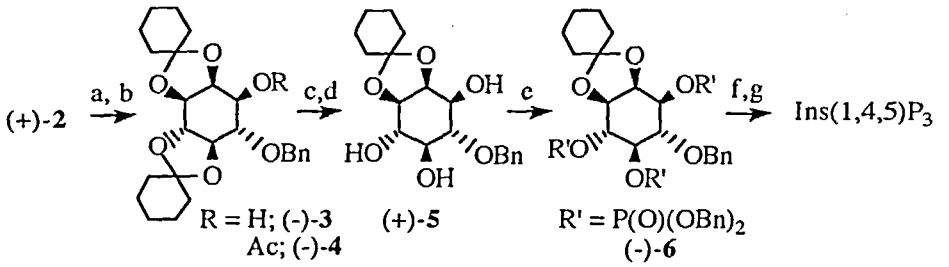
the second-messenger role of inositol phosphates in Ca^{2+} signaling constitutes one of our research foci. In this account, we report the systematic synthesis of phosphoinositol congeners and their interactions with the Ins(1,4,5)P_3 receptor.

CHEMOENZYMATIC SYNTHESIS OF INOSITOL PHOSPHATES

Our key strategy employed a pair of enantiomerically active 1,2:5,6-dicyclohexylidene-*myo*-inositols (**2**) as common precursors to the target molecules, which was prepared by a facile enzymatic method. The 4-butyryl monoester [(±)-**1**] was subjected to enantiospecific hydrolysis by porcine pancreatic lipase, which gave both product [(-)-**2**] and substrate [(-)-**1**] fractions with satisfactory optical purity (e.e. ≥ 0.98) after recrystallization.



The synthetic utility of **2** is illustrated by the example of Ins(1,4,5)P_3 synthesis.



a) *n*-Bu_2SnO, BnBr, CsF; b) Ac_2O, DMAP; c) $TsOH/CH_2Cl_2$; d) 1 *N* KOH/MeOH
e) $(BnO)_2P$-N(*i*Pr)$_2$, 1-*H*-tetrazole, MCPBA; f) Pd/C, H_2/95% EtOH; g) AcOH

Both enantiomerically active **2** allow the synthesis of eleven D-*myo*-inositol phosphates in fair yields, which included Ins(1,4)P_2, Ins(4,5)P_2, Ins(1,3,4)P_3, Ins(1,4,5)P_3, Ins(1,5,6)P_3, Ins(1,2,5,6)P_4, Ins(1,3,4,5)P_4, Ins(1,3,4,6)P_4, Ins(1,4,5,6)P_4, Ins(3,4,5,6)P_4, Ins(1,3,4,5,6)P_5.

INOSITOL PHOSPHATE-INDUCED CALCIUM RELEASE

Ca^{2+}-loaded rat brain microsomes were treated with individual inositol phosphates at 37 °C, and the released Ca^{2+} was monitored by bulk fluorimetry using Fura-2 as an indicator. Of the 12 phosphoinositols examined (the aforementioned 11 synthetic molecules and glycerophospho-D-*myo*-inositol 4,5-bisphosphate [GroPIns(4,5)P_2; purchased from Sigma], Ins(1,4,5)P_3, GroPIns(4,5)P_2, Ins(1,3,4,6)P_4, Ins(1,3,4,5)P_4, Ins(1,4,5,6)P_4, and Ins(4,5)P_2 exhibited Ca^{2+}-mobilizing activity in a dose-dependent manner (Figure 2), with apparent EC_{50} values of 0.13, 1.3, 4.4, 8.2, 11.2, and 60 μM, respectively. Other inositol phosphates including Ins(1,4)P_2, Ins(1,5,6)P_3, Ins(1,3,4)P_3, Ins(3,4,5,6)P_4, Ins(1,2,5,6)P_4, and Ins(1,3,4,5,6)P_5 failed to exert appreciable Ca^{2+} release from the microsomal preparation, even at concentrations up to 100 μM.

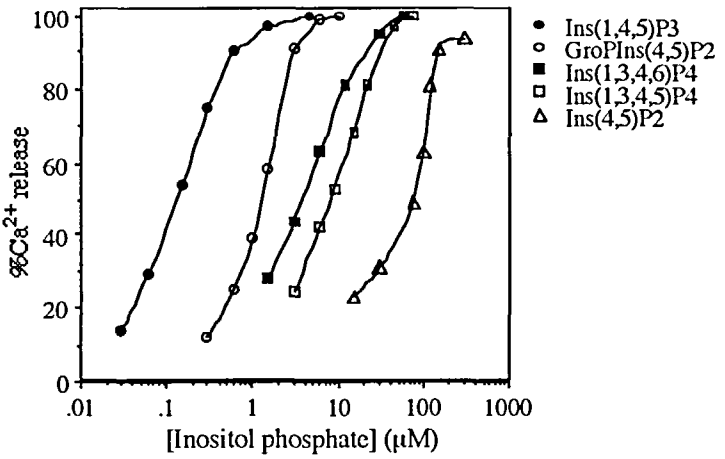


Figure 2 Inositol phosphate-induced Ca^{2+} release from rat brain microsomes.

BINDING AFFINITY OF INOSITOL PHOSPHATES WITH THE INS(1,4,5)P_3 RECEPTOR

To assess the binding of inositol phosphates to the Ins(1,4,5)P_3 receptor, displacement of specific [^{3}H]Ins(1,4,5)P_3 binding was carried out using rat cerebellar membrane preparations. According to the displacement curves (not shown), the mean dissociation constants (K_d) for individual inositol phosphates were determined as follows (n = 3): Ins(1,4,5)P_3, 0.028 μM; GroPIns(4,5)P_2, 0.92 μM; Ins(1,3,4,5)P_4, 1.4 μM; Ins(1,4,5,6)P_4, 2.1 μM; Ins(1,3,4,6)P_4, 2.2 μM; Ins(4,5)P_2, 24 μM; Ins(1,3,4,5,6)P_5, 40 μM; Ins(3,4,5,6)P_4, 56 μM; Ins(1,2,5,6)P_4, 57 μM; Ins(1,3,4)P_3, 146 μM; Ins(1,4)P_2, 217 μM; Ins (1,5,6)P_3, 454 μM. For the inositol phosphates capable of effecting Ca^{2+} mobilization, the relative potency of inhibiting [^{3}H]Ins(1,4,5)P_3 binding to the receptor paralleled the order of the EC_{50} values.

LIGAND RECOGNITION AT THE INS(1,4,5)P_3 RECEPTOR

Analysis of the structures of Ca^{2+}-mobilizing inositol phosphates indicates that all these molecules assume conformations sharing or mimicking the structural features of the 4,5-bisphosphate 6-hydroxy and 1-phosphate motifs of Ins(1,4,5)P_3 (Figure 3)

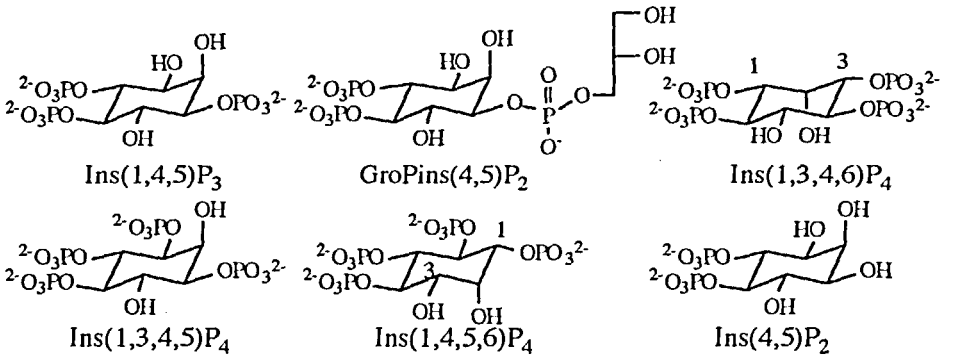


Figure 3 Structures of some inositol phosphates capable of eliciting Ca^{2+} release

On the basis of this finding, we propose a binding model to account for ligand recognition at the Ins(1,4,5)P_3 receptor (Figure 4). The binding site is presumably composed of two domains. The anchoring domain interacts with the 4,5-bisphosphate 6-hydroxy motif, attributing to the Ca^{2+}-mobilizing activity. The auxiliary domain exerts long-range electrostatic interactions with the 1-phosphate group, which enhances the binding affinity. The stereochemical requirement for this phosphate recognition is, however, less stringent. The biochemical implication of the cross-reactivity of the Ins(1,4,5)P_3 receptor with a number of inositol phosphates besides Ins(1,4,5)P_3 remains unclear.

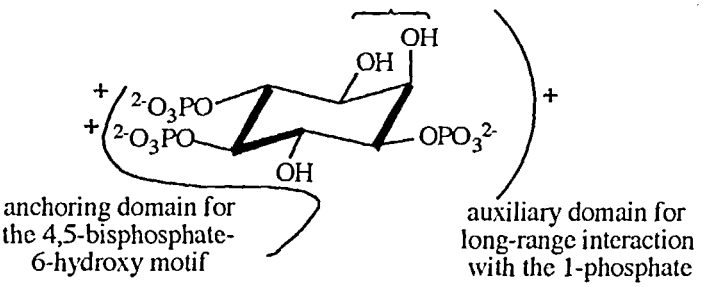


Figure 4. Ligand recognition at the Ins(1,4,5)P_3-binding site.

Reference

D.-M. Gou, Y.-C. Liu, & C.-S. Chen (1992) *Carbohydr. Res.* 234, 51-64.

P.-J. Lu, D.-M. Gou, W.-R. Shieh, & C.-S. Chen (1994) *Biochemistry* 33, 11586-11597.