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# Phosphorus, Sulfur, and Silicon and the Related Elements

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#### MODULATION OF THE INOSITOL 1,4,5-TRISPHOSPHATE RECEPTOR BY INOSITOL PHOSPHATES

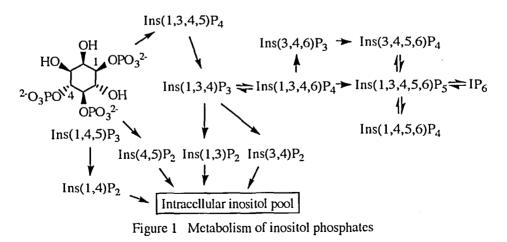
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Abstract The mode of recognition at the binding site of the  $Ins(1,4,5)P_3$  receptor was assessed by examining the structure-activity relationships of different Ca<sup>2+</sup>-mobilizing inositol phosphates.

*Key Words:* the Ins(1,4,5)P<sub>3</sub> 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<sup>2+</sup> 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).

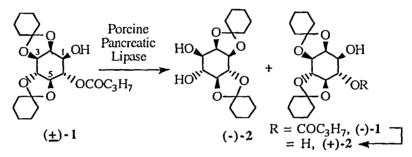


The rich diversity of phosphoinositols generated from such a complex metabolic network implies the physiological relevance of these molecules. Thus, examination of

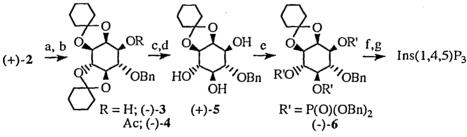
the second-messenger role of inositol phosphates in  $Ca^{2+}$  signaling constitutes one of our research foci. In this account, we report the systematic synthesis of phosphoinositol congeners and their interactions with the Ins(1,4,5)P<sub>3</sub> receptor.

## CHEMOENZYMATIC SYNTHESIS OF INOSITOL PHOSPHATES

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



The synthetic utility of 2 is illustrated by the example of Ins(1,4,5)P3 synthesis.



a) *n*-Bu<sub>2</sub>SnO, BnBr, CsF; b) Ac<sub>2</sub>O, DMAP; c) TsOH/CH<sub>2</sub>Cl<sub>2</sub>; d) 1 N KOH/McOH e) (BnO)<sub>2</sub>P-N(*i*Pr)<sub>2</sub>, 1-*H*-tetrazole, MCPBA; f) Pd/C, H<sub>2</sub>/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<sup>2+</sup>-loaded rat brain microsomes were treated with individual inositol phosphates at 37 °C, and the released Ca<sup>2+</sup> 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<sub>2</sub>; purchased from Sigma], Ins(1,4,5)P<sub>3</sub>, GroPIns(4,5)P<sub>2</sub>, Ins(1,3,4,6)P<sub>4</sub>, Ins(1,3,4,5)P<sub>4</sub>, Ins(1,4,5,6)P<sub>4</sub>, and Ins(4,5)P<sub>2</sub> exhibited Ca<sup>2+</sup>-mobilizing activity in a dose-dependent manner (Figure 2), with apparent EC<sub>50</sub> values of 0.13, 1.3, 4.4, 8.2, 11.2, and 60  $\mu$ M, respectively. Other inositol phosphates including Ins(1,4)P<sub>2</sub>, Ins(1,5,6)P<sub>3</sub>, Ins(1,3,4)P<sub>3</sub>, Ins(3,4,5,6)P<sub>4</sub>, Ins(1,2,5,6)P<sub>4</sub>, and Ins(1,3,4,5,6)P<sub>5</sub> failed to exert appreciable Ca<sup>2+</sup> release from the microsomal preparation, even at concentrations up to 100  $\mu$ M.

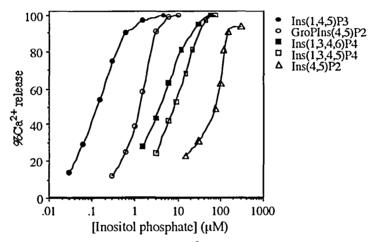


Figure 2 Inositol phosphate-induced Ca<sup>2+</sup> release from rat brain microsomes.

## BINDING AFFINITY OF INOSITOL PHOSPHATES WITH THE INS(1,4,5)P<sub>3</sub> RECEPTOR

To assess the binding of inositol phosphates to the  $Ins(1,4,5)P_3$  receptor, displacement of specific [<sup>3</sup>H]Ins(1,4,5)P<sub>3</sub> binding was carried out using rat cerebellar membrane preparations. According to the displacement curves (not shown), the mean dissociation constants (K<sub>d</sub>) for individual inositol phosphates were determined as follows (n = 3):  $Ins(1,4,5)P_3$ , 0.028  $\mu$ M; GroPIns(4,5)P\_2, 0.92  $\mu$ M; Ins(1,3,4,5)P\_4, 1.4  $\mu$ M;  $Ins(1,4,5,6)P_4$ , 2.1  $\mu$ M; Ins(1,3,4,6)P\_4, 2.2  $\mu$ M; Ins(4,5)P\_2, 24  $\mu$ M; Ins(1,3,4,5,6)P\_5, 40  $\mu$ M; Ins(3,4,5,6)P\_4, 56  $\mu$ M; Ins(1,2,5,6)P\_4, 57  $\mu$ M; Ins(1,3,4)P\_3, 146  $\mu$ M; Ins(1,4)P\_2, 217  $\mu$ M; Ins (1,5,6)P\_3, 454  $\mu$ M. For the inositol phosphates capable of effecting Ca<sup>2+</sup> mobilization, the relative potency of inhibiting [<sup>3</sup>H]Ins(1,4,5)P\_3 binding to the receptor paralleled the order of the EC<sub>50</sub> values.

#### LIGAND RECOGNITION AT THE INS(1,4,5)P3 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<sub>3</sub> (Figure 3)

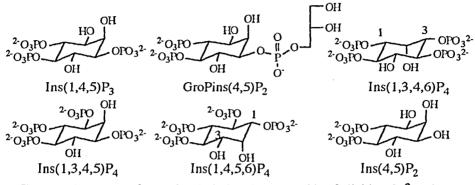


Figure 3 Structures of some inositol phosphates capable of eliciting Ca2+ release

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<sup>2+</sup>-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<sub>3</sub> receptor with a number of inositol phosphates besides Ins(1,4,5)P<sub>3</sub> remains unclear.

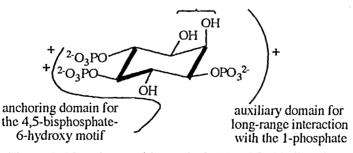


Figure 4. Ligand recognition at the Ins(1,4,5)P3-binding site.

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