sirius fine chemicals

SiChem manual

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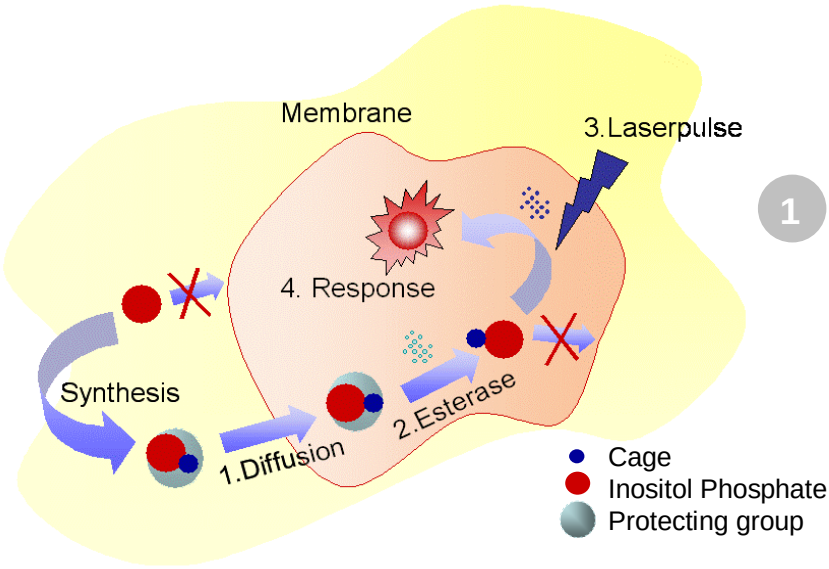
Photoactivatable and Membrane permeant Ins(1,4,5)P3 - caged IP3

Membrane-permeant derivatives of inositol polyphosphates require the intracellular enzymatic hydrolysis of several protecting groups, for instance of acyloxymethyl esters, in order to generate the biologically active compound. The highly complicated kinetics of these biochemical steps may lead to unphysiological effects. The physiological signal usually appears to be very rapid. The photolysis of membrane-permeant caged derivatives of $Ins(1,4,5)P_3$ mimic fast intracellular responses. In an initial step cells are loaded with the caged $Ins(1,4,5)P_3$/PM derivative. Within 30-180 minutes all bioactivatable protecting groups remove, generating caged inositol polyphosphate. The cage is known to prevent biological activity when placed at the right position, in this case the 6-hydroxy-group.



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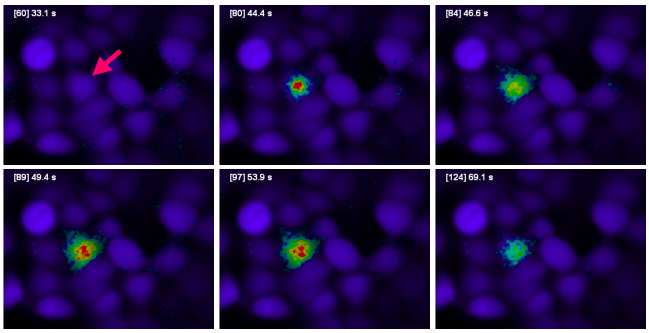
The photochemical destruction of the cage (~360 nm) releases active $Ins(1,4,5)P_3$ within a few seconds, thus mimicking the rapid responses of the receptor / phospholipase C signaling system in the cell (1).

Experiments

Since this approach does not directly trigger other signaling events, for instance PKC activation after receptor-mediated diacylglycerol (DAG) formation, membrane-permeant derivatives of signaling molecules are able to help dissecting signaling pathways. In the shown experiment HeLa cells were co-loadedwith 2 µM caged $Ins(1,4,5)P_3$/PM [cag-iso-2-145] and Oregon Green 488 BAPTA-1AM. All compounds were loaded for 80 min at room temperature. Stimulus was 1 burst of 5 UV flashes (300-400 nm bandwidth) delivered 42 sec after the onset of the recording: $[Ca^{2+}]_i$ raised ! (2)



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Experiments performed by
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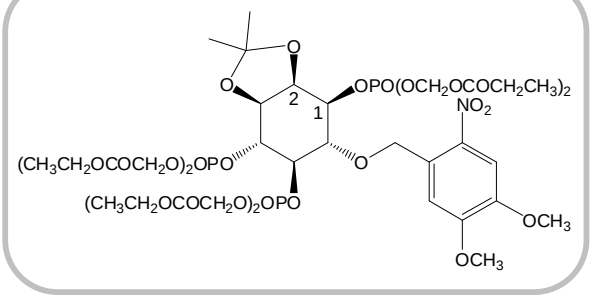
Prof. Fabio Mammano at the Venetian
Institute of Molecular Medicine – Padua
University, Italy, http://www.vimm.it



D-2,3-*O*-Isopropylidene-6-*O*-(2-nitro-4,5-dimethoxy)benzyl-*myo*-Inositol 1,4,5-trisphosphate-Hexakis(propionoxymethyl) Ester

Product No.: cag-iso-2-145
Formula: $C_{42}\ H_{64}\ N\ O_{31}\ P_3$
MW: 1171.27

Preparation

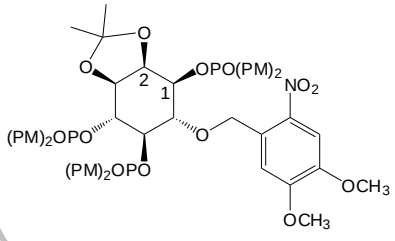
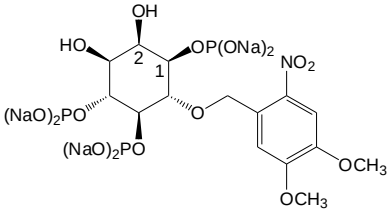
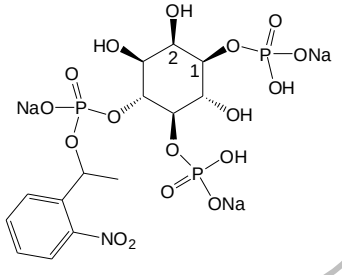
It is probably a good idea to aliquot the sample. The compound is soluble in CH_2Cl_2 or DMSO, which evaporates instantly under reduced pressure. The evaporation vessel should be filled with argon (better) or nitrogen afterwards. The compound is sensitive to water on a longer time scale. Therefore, please store the compound in substance or in dry DMSO (for not longer than 2 weeks) at –20 °C or below. The freezing process should be performed very quickly (-80 °C), not just in the freezer. For incubations, dissolve an aliquot of the cell penetrating compound in dry DMSO.

Take out a small amount (e.g. 1 µl) and mix with same amount of Pluronic® F127 in DMSO (10%).
To this mixture add 100 µl of the **serum-free cell supernatant**, mix thoroughly with a pipette and immediately add back to the cells. The final DMSO concentration in the experiment should not exceed 0.5%. The final concentration of caged-iso-Ins(1,4,5)P_3/PM should be in the **1-3 µM** range, depending on the cell type. For calcium measurements after de-caging use one of the standard calcium sensors. **Incubation** in the dark at room temperature for the cell penetrating compound and the calcium sensor should be **30-120 min.** Subsequently, it is possible to return to different buffers (with serum, if necessary). If buffer is not changed, **calcium levels** can be measured **within 5-10 min**. If buffers are changed, a longer adaptation phase (30 min) is recommended. To **un-cage** caged-iso-Ins(1,4,5)P_3/PM, scan cells once with an excitation around **360 nm** of an argon-ion UV laser or another UV light source

Summary:

- stock solution in CH_2Cl_2 or DMSO (storage: 2 weeks, -20 °C)
- final concentration of ciInsP_3/PM: 1-3 µM
- incubation: 30-120 min.
- uncage cagedInsP_3/PM: with an argon-ion UV laser using the 345–355 nm line

Other caged Inositol Phosphates

[cag-iso-2-145]	[cag-6-145]	[cag-0-145]
caged-InsP_3-DMNB Membrane-permeant and photolabile derivate of Ins(1,4,5)P_3 with DMNB (D-23-O-Isopropylidene-6-O-(2-nitro-4.5-dimethoxy)	caged-InsP_3-DMNB Photolabile derivate of Ins(1,4,5)P_3 with the same caged group as the membrane-permeant derivative [cag-iso-2-145]. It is photolyzed with UV light about three times more efficiently than the widely used D-*myo*-Inositol Trisphosphate-NPE (P^4-1-(2-Nitrophenyl)ethyl Ester) [cag-0-145]	caged-InsP_3-NPE Photolabile derivate of Ins(1,4,5)P_3 with NPE (P^4-1-(2-Nitrophenyl)ethyl Ester)
10 * 10 µg / 1 * 100 µg	1 * 100 µg	1 * 100 µg
		

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