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Interaction of *myo*-inositol hexakisphosphate with alkali and alkaline earth metal ions: Spectroscopic, potentiometric and theoretical studies

Julia Torres [a], Nicolás Veiga [a], Jorge S. Gancheff [a], Sixto Domínguez [b], Alfredo Mederos [b], Markku Sundberg [c], Agustín Sánchez [d], Jorge Castiglioni [e], Alvaro Díaz [f], Carlos Kremer [a,*]

[a] *Cátedra de Química Inorgánica, Departamento Estrella Campos, Facultad de Química, Universidad de la República, CC 1157 Montevideo, Uruguay*
[b] *Departamento de Química Inorgánica, Universidad de La Laguna, Tenerife, Canary Islands, Spain*
[c] *Department of Chemistry, University of Helsinki, PO Box 55, FIN-00014, Finland*
[d] *Departamento de Química Inorgánica, Facultade de Farmacia, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Galicia, Spain*
[e] *LAFIDESU, Facultad de Química, Universidad de la República, CC 1157 Montevideo, Uruguay*
[f] *Cátedra de Inmunología, Facultad de Química/Ciencias, Universidad de la República, Avenida Alfredo Navarro 3051, CP 11600 Montevideo, Uruguay*

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Abstract

myo-Inositol hexakisphosphate (InsP_6) interacts strongly with cations, forming soluble complexes and solids, both of which are biologically relevant. We have recently reported advances in this area, showing the importance of the interaction with Mg^{2+} and Ca^{2+}. We now extend the data to Sr^{2+} and Ba^{2+}, which behaved similarly to Mg^{2+} and Ca^{2+}, but giving even less soluble systems. We also describe the interactions with Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} in a non-interacting medium: these depended strongly on cation charge but only weakly on ionic radius within each series. In addition, we probed structural aspects of InsP_6 compounds with alkaline earth cations, by means of IR and Raman spectroscopy of the solids and computational calculations on the 1:1 complexes of Mg^{2+}. Both approaches suggested direct and multidentate phosphate-metal coordination. In $[Mg(H_2L)(H_2O)_3]^{8-}$, three oxygen atoms from two phosphate groups and three water molecules were predicted to form a roughly octahedral coordination shell; alternate or vicinal phosphate groups were predicted to chelate Mg^{2+} depending on whether InsP_6 is in the 5-axial/1-equatorial or the 1-axial/5-equatorial conformation, respectively.

Keywords: Inositol hexakisphosphate; Inositol polyphosphates; Alkali cations; Alkaline earth cations

1. Introduction

myo-Inositol hexakisphosphate (InsP_6, $H_{12}L$) is an ubiquitous and abundant molecule in eukaryotic cells, whose biological functions are incompletely understood (reviewed in [1–4]). InsP_6 is necessary (in mammalian cells) for the repair of double-stranded breaks in DNA and for RNA editing, and (in yeasts) for mRNA export from the nucleus. Neither in mammalian nor in yeast cells do the overall levels of this metabolite fluctuate acutely; instead, InsP_6 seems to participate in the processes mentioned as a cofactor or structural element permanently incorporated into the enzymatic machinery. In contrast, InsP_6 levels can fluctuate and have signalling roles in plant cells. In animals, yeasts and Dictyostelids, InsP_6 is also the precursor of inositol pyrophosphates, which are emerging as fast-turnover, conventional, intracellular messengers (reviewed in [5]). The set of functions mentioned correspond to the nuclear-cytosolic pool of InsP_6, thought to exist in all eukaryotic cells and to be constituted by soluble InsP_6 (perhaps together with

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* Corresponding author. Tel.: +598 29249739; fax: +598 29241906.
E-mail address: ckremer@fq.edu.uy (C. Kremer).

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some membrane-bound compound). In addition, some eukaryotic cells have vesicular and even extracellular pools of InsP_6 [6–8]. Notably, vacuolar InsP_6 and its associated cations act as nutritional reserves in plant storage tissues [9].

The biology and biochemistry of InsP_6 is strongly intertwined with its chemistry of interaction with multivalent cations. Since the early reports [10–29] it was clear that this chemistry comprises two different aspects: the formation of soluble species exhibiting 1:1 stoichiometry (dominant when InsP_6 is in excess) and the formation of sparingly soluble solids (dominant under conditions of metal excess). Although it is the second aspect that is biologically most relevant (given that in mammals, intracellular concentrations of InsP_6 are in the tens of µM range; reviewed in [1,30]), any rigorous treatment of the matter requires the inclusion of the solution complexation equilibria. In addition, InsP_6 interacts to a significant extent with alkali metal cations [31–37], and hence biologically useful descriptions need to incorporate physiological concentrations of these.

In the first of our previous studies [38], we reported a detailed study of the interaction of InsP_6 with multivalent cations, under ligand excess, at $I = 0.15$ M $NaClO_4$ and 37.0 °C. Three major chemical conclusions were drawn from this work. First, the species formed always contain 1 mol of InsP_6 per mol of cation, for both divalent (Mg, Ca, Mn, Fe, Co, Ni, Cu, Zn, Cd) and trivalent (Fe, Al) cations. Second, the stability constants of the 1:1 species are very large, the complexation capability of InsP_6 being comparable to that of EDTA. Consistent with primarily electrostatic interactions, constants are larger for trivalent than for divalent cations. Notwithstanding, interactions are detectable even for Na^+; data from a limited preliminary study in non-interacting medium allowed to predict that, at physiological concentrations (60 µM), pH 7.4, 37.0 °C, and in the presence of 0.15 M Na^+, InsP_6 exists predominantly as $[Na_4(H_3L)]^{5-}$ and $[Na_3(H_4L)]^{5-}$ [38]. The third conclusion is that Mg^{2+} constitutes a special case among the divalent cations, since a neutral pentamagnesium complex $[Mg_5(H_2L)]$ is detected in solution besides the 1:1 species when a moderate cation excess is employed. This 5:1 soluble species appears to owe its existence to a especially large window of solubility of the solid, which also has a 5:1 stoichiometry. This solubility window (probably together with association with proteins) explains that InsP_6 can be soluble in the cytosol and nucleus of mammalian cells, where it coexists with mM-range concentrations of Mg^{2+}. Indeed, when a physiological concentration of interaction-available Mg^{2+} (0.8 mM) is included alongside the conditions detailed above in the context of its interactions with Na^+, InsP_6 is predicted to exist almost completely as $[Mg_5(H_2L)]$.

In our very recent second paper [39], we extended our study to the formation of the Ca solid, which also has a 5:1 stoichiometry, and quantified the solubilities of both the Ca and Mg compounds. These data show that, again in 0.15 M $NaClO_4$ and at 37.0 °C, $[Mg_5(H_2L)]$ starts to precipitate when it reaches 49 µM, irrespective of pH. Meanwhile, the calcium solid is much less soluble, lacking a detectable window of solubility as 5:1 complex. This determines that in Ca^{2+}-rich media such as the extracellular medium of animals, InsP_6 exists only as the Ca solid, as we have experimentally verified in the larval *Echinococcus granulosus* system [6]. Although a rigorous chemical description of the InsP_6 deposits in plant storage tissues is lacking, we have also pointed out [6] that sequential precipitation of the increasingly soluble compounds of InsP_6 with Ca, Mg, and K (this latter as yet undescribed formally) might explain the composition of the major type of vacuolar phytate deposit found in plants [9,40,41].

On the basis of the results summarized above, it is clear that a complete description of the chemistry of InsP_6 with cations should include: the speciation study under ligand excess, the characterization of the solid phases formed under metal excess, and the determination of the solubility products of the solids. The biologically most pressing points having been already advanced in the earlier papers [38,39], the present article includes:

(i) the determination of the stability constants (37.0 °C, $I = 0.15$ M $NaClO_4$ or NMe_4Cl) of the complexes formed by InsP_6 with M^+ (Li, Na, K) and M^{2+} (Mg, Ca, Sr, Ba),
(ii) the synthesis and chemical characterization of the solids obtained under M^{2+} molar excess for M = Sr and Ba, and
(iii) the determination of the solubility products for the $[M_5(H_2L)]\cdot xH_2O$ species (M = Sr, Ba),

therefore completing the coverage of the aspects mentioned for the alkali and alkaline earth metal ions (except for the study of the highly soluble solids with alkali metal ions). In addition to basic bioinorganic knowledge, the article contributes to certain points of immediate biological relevance, since K^+ (and not Na^+) is the major intracellular alkali cation, and Li^+ is relevant from the pharmacological point of view.

In addition, the article includes:

(iv) a structural study of the solid species $[M_5(H_2L)]\cdot xH_2O$ (M = Mg, Ca, Sr, Ba) by NMR, IR, and Raman spectroscopy, and a computational approximation to the structure of selected soluble 1:1 complexes.

The structural aspect is also important both chemically and biologically. As explained above, the biologically most relevant forms of "protein-free" InsP_6 are soluble $[Mg_5(H_2L)]$ and the Mg and Ca solids. However, no data are so far available on the structures of these compounds, a gap that hampers obtaining biological insights into them.