

A Crystallographically Characterized Nine-Coordinate Calcium–Phosphocitrate Complex as Calcification Inhibitor in Vivo

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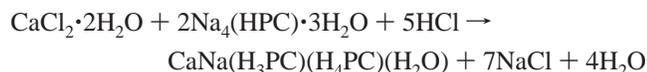
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Calcium-containing crystal deposition diseases represent a group of clinically heterogeneous calcific diseases that are a significant source of morbidity.¹ Phosphorylated carboxylic acids are powerful inhibitors of biological crystallization as it relates to those diseases.² Phosphocitrate (PC), a naturally occurring compound (Chart 1),³ is particularly potent. It has been demonstrated to inhibit the transformation of calcium phosphate to hydroxyapatite (Ca₅(PO₄)₃(OH)),⁴ the deposition of calcium oxalates,⁵ the crystallization of octacalcium phosphate (Ca₈(HPO₄)₂(PO₄)₄·5H₂O),⁶ and calcium pyrophosphate (Ca₂P₂O₇·2H₂O),⁷ and the formation of struvite (Mg(NH₄)(PO₄)·6H₂O) in vivo.⁸ PC has also expressed inhibitory activity against the formation of scaling Ca salts, such as calcite (CaCO₃) and gypsum (CaSO₄·2H₂O), related to industrial water treatment.⁹ In addition, allied to these later actions, PC has been noted to prevent corrosion of carbon steel surfaces.⁹ Overall then, the compound attracts keen interest from the viewpoint of its nontoxic nature¹⁰ and potential to influence biomineralization in many diverse biological fields.

In this paper we describe the preparation and crystal and molecular structure of a polymeric mixed salt of PC, namely [CaNa(PC)₂(H₂O)]_n (CaNaPC), and its improved calcification inhibition properties compared to its precursor, NaPC.¹¹

CaNaPC forms by the reaction of NaPC and Ca²⁺ at pH ~2 according to the balanced equation (protons on PC also shown):



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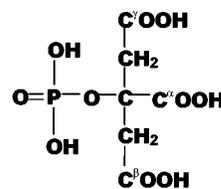
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Chart 1. Schematic Structure of Phosphocitric Acid (H₅PC)



The structure of CaNaPC¹² (Figure 1) can be described as polymeric in nature with Ca(PC)₂(H₂O) “monomers” linked through Na⁺ bridges.

Ca is coordinated by four phosphate, four carbonyl, and one water O-atoms defining an irregular polyhedron. Coordination number nine for Ca is rather rare.¹³ In that regard, the unexpected presence of a coordinated H₂O is the result of the strain imposed by the PC ligand on the coordination geometry, making a wide site available to H₂O. Two biologically relevant examples of nine-coordinate Ca are β-calcium-pyrophosphate¹⁴ and hydroxyapatite.¹⁵ An intriguing feature in the structure of CaNaPC is the short distance of 2.477(1) Å between Ca and the ester O from C–O–PO₃H₂. For comparison, the Ca–O(pyrophosphate ester) distance in β-Ca₂(P₂O₇) is 2.855 Å. Interestingly, this is consistent with the apparent resistance of the P–O–C moiety to hydrolysis in an acidic environment, suggesting that strong calcium coordination exerts a prominent protective effect on the overall molecule. The carbonyl oxygens are coordinated to the Ca center with Ca–O distances in the 2.446(2)–2.586(2) Å range, much shorter than those in Ca hydrogen citrate trihydrate (2.37–2.49 Å).¹⁶ Similarly, the Ca–O(PO₃H) distance is 2.527(2) Å, much longer than Ca–O distances in related complexes (2.3–2.4 Å).¹⁷ Ca–O distances become elongated as coordination number increases. Ca–O distances in CaNaPC are consistent with these observations. All –COOH groups are protonated due to the low pH of preparation. There are three dissociated protons per two PC molecules,¹⁸ all coming from –PO₃H₂. Unexpectedly, the second proton from –PO₃H is dissociated before that from α-COOH and is involved in a short hydrogen bond (2.453(3) Å)

(11) In this paper the abbreviation “PC” is used with no specific reference to proton content, unless otherwise noted. Synthesis and characterization of NaPC, as the tetrakis-deprotonated Na₄(HPC)·3H₂O, has been described (Williams, G.; Sallis, J. D. *Anal. Biochem.* **1980**, *102*, 365. Tew, W. P.; Mahle, C.; Benavides, J.; Howard, J. E.; Lehninger, A. L. *Biochemistry* **1980**, *19*, 1983). For the synthesis of CaNaPC, NaPC (3.24 g, 7.83 mmol) was dissolved in 200 mL of distilled water. CaCl₂·2H₂O (2.86 g, 19.64 mmol; this amount of Ca²⁺ gave the highest yields) was added gradually as a solid under stirring. The pH started decreasing and some cloudiness formed. Final pH was adjusted to ~2 with dilute HCl. The solution was then taken to dryness with a Rotovap. Excess Ca²⁺ and Na⁺ were removed by washing briefly with distilled water. Yield 2.5 g (70%). Product purity was verified by ICP of an aqueous solution of the material. FT-IR spectrum (KBr disks): ν_{C=O} 1717, 1636 cm⁻¹, ν_{O–H} 3573, 3496 cm⁻¹, ν_{P=O}(asym) 1260, 1230 cm⁻¹, and ν_{P=O}(sym) 1090, 1075 cm⁻¹. Our attempts to prepare crystalline CaPC salts at higher pH have thus far failed due to rapid salt precipitation.

(12) Single crystals of CaNaPC were grown by slow evaporation of a concentrated aqueous solution of the salt prepared as above. They are colorless, monoclinic (space group C2/c), with a = 22.331(3) Å, b = 7.966(1) Å, c = 13.233(2) Å, β = 107.877(2)°, V = 2240.2(5) Å³, Z = 8, FW = 311.13, and d_{calc} = 1.845 g/cm³. Intensity data were collected on a CCD SMART diffractometer with Mo Kα radiation. A total of 4751 reflections were measured (1612 unique), 1478 with I > 2σ(I) used in the structure refinement by full-matrix least-squares techniques (173 parameters). Final R indices: R₁ = 0.0277, wR₂ = 0.0753, and GoF = 1.072 (for all reflections R₁ = 0.0303 and wR₂ = 0.0770).

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