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From crystalline to amorphous calcium pyrophosphates: A solid state Nuclear Magnetic Resonance perspective

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ABSTRACT

Hydrated calcium pyrophosphates (CPP, $Ca_2P_2O_7{\cdot}nH_2O$) are a fundamental family of materials among osteoarticular pathologic calcifications. In this contribution, a comprehensive multinuclear NMR (Nuclear Magnetic Resonance) study of four crystalline and two amorphous phases of this family is presented. ^{1}H, ^{31}P and ^{43}Ca MAS (Magic Angle Spinning) NMR spectra were recorded, leading to informative fingerprints characterizing each compound. In particular, different ^{1}H and ^{43}Ca solid state NMR signatures were observed for the amorphous phases, depending on the synthetic procedure used. The NMR parameters of the crystalline phases were determined using the GIPAW (Gauge Including Projected Augmented Wave) DFT approach, based on first-principles calculations. In some cases, relaxed structures were found to improve the agreement between experimental and calculated values, demonstrating the importance of proton positions and pyrophosphate local geometry in this particular NMR crystallography approach. Such calculations serve as a basis for the future *ab initio* modeling of the amorphous CPP phases.

Statement of significance

The general concept of NMR crystallography is applied to the detailed study of calcium pyrophosphates (CPP), whether hydrated or not, and whether crystalline or amorphous. CPP are a fundamental family of materials among osteoarticular pathologic calcifications. Their prevalence increases with age, impacting on 17.5% of the population after the age of 80. They are frequently involved or associated with acute articular arthritis such as pseudogout. Current treatments are mainly directed at relieving the symptoms of joint inflammation but not at inhibiting CPP formation nor at dissolving these crystals. The combination of advanced NMR techniques, modeling and DFT based calculation of NMR parameters allows new original insights in the detailed structural description of this important class of biomaterials.

1. Introduction

Crystalline calcium pyrophosphate dihydrates (CPPD, $Ca_2P_2O_7{\cdot}2H_2O$) are among the most common forms of pathologic articular minerals: their prevalence increases with age, impacting on 17.5% of the population after the age of 80 [1]. Although often asymptomatic, they are frequently involved or associated with acute articular arthritis such as pseudogout, and, more rarely, with chronic polyarthritis and destructive arthropathy; current treatments are mainly directed at relieving the symptoms of joint inflammation but not at inhibiting calcium pyrophosphate (CPP) formation nor at dissolving these crystals [2–4].

CPP have been identified *in vivo* as two polymorphs of CPPD [5]: a triclinic form with a known structure [6], and a monoclinic form with a recently solved structure [7] (respectively denoted as t-CPPD and m-CPPD). Other crystalline forms of hydrated calcium pyrophosphates have been synthesized *in vitro* and characterized,

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including a dimorphic monoclinic tetrahydrate (CPPT: $Ca_2P_2O_7{\cdot}4H_2O$), referred to as m-CPPT α and m-CPPT β [8,9]. Recently, Gras et al. [7] performed a systematic investigation of the synthesis of pure hydrated calcium pyrophosphates. They described the pH and temperature conditions leading to the formation of m-CPPT β, t-CPPD and m-CPPD [10], as well as the identification of a new monohydrated calcium pyrophosphate phase exhibiting monoclinic symmetry, referred to as m-CPPM (CPPM: $Ca_2P_2O_7{\cdot}H_2O$) [11], and an unreferenced highly metastable trihydrated monoclinic calcium pyrophosphate phase derived from the structure of m-CPPT β [12]. The preparation of amorphous phases of biological interest, noted a-CPP ($Ca_2P_2O_7{\cdot}nH_2O$), has also been reported by Slater et al. [13] and Gras [10], and it was found that these phases are particularly stable compared to amorphous calcium orthophosphate and amorphous calcium carbonate.

Several characterizations have been performed on all the hydrated calcium pyrophosphate phases mentioned above by several approaches, including powder XRD (X Ray diffraction) and vibrational spectroscopies, providing information on the configuration of the pyrophosphate groups [10]. It was observed that pyrophosphate ions in CPP phases have a wide range of P–O–P angles (between 123.1° and 134.1°) [8,9]. This angle is important in understanding the relationship between the various CPP forms and their stability and transformation ability. Developing complementary tools for the characterization of hydrated calcium pyrophosphates is of particular interest, especially to understand the structure of phases like m-CPPD, for which the positioning of protons may be very difficult based exclusively on X-ray powder diffraction data, particularly considering that single crystals suitable for diffraction structure resolution are not yet available. Indeed, this phase has the highest inflammatory potential of all CPP phases, and it would be of interest to determine its structure in detail in order to understand the inflammation mechanism, which is possibly based on rupture of lysosome phospholipid membranes induced by pyrophosphate groups on the surface of the crystals [14–17].

Solid state NMR is a technique which is attracting increasing attention for the study of synthetic and natural biomaterials [18] including calcium phosphate phases [19–27]. Indeed, solid state NMR can provide detailed atomic-scale information on the local structure around nuclei like ^{31}P, even in disordered and amorphous phases, and is therefore highly complementary to other analytical tools like XRD and IR (Infra Red) or Raman spectroscopies. NMR studies of calcium pyrophosphate phases have been very limited to date. To the best of our knowledge, ^{31}P NMR has only been applied to the characterization of the crystalline α- and β-$Ca_2P_2O_7$ anhydrous phases, and of a hydrated amorphous calcium pyrophosphate of composition $\sim Ca_2P_2O_7{\cdot}4H_2O$ [13,28]. In the latter case, the hydrolysis of the P–O–P bridge upon heat treatment was demonstrated using ^{1}H MAS, ^{31}P MAS and ^{1}H–^{31}P cross-polarization (CP) MAS NMR experiments. With regards to ^{43}Ca NMR, only the anhydrous α-$Ca_2P_2O_7$ phase has been analyzed to date [29], showing that the two crystallographically-inequivalent Ca sites can be unambiguously resolved at 14.1 T. Although ^{43}Ca is a more challenging nucleus than ^{31}P [30,31] given its quadrupolar nature [32], low natural abundance (0.14%) and small magnetic moment (leading it to be a member of the group of so-called low-γ nuclei) [33], recent studies have shown that it can be very sensitive to subtle changes in Ca local environments [34–36]. Finally, even more challenging isotopes like oxygen-17 which usually require isotopic enrichment have been completely neglected so far.

The purpose of this study is to demonstrate, using a combined experimental–computational approach, how solid state NMR can be used for the structural investigation of calcium pyrophosphate phases, whether hydrated or anhydrous, and whether crystalline or amorphous. For this purpose, the ^{31}P, ^{43}Ca and ^{1}H MAS NMR spectra of a series of crystalline CPP phases (m-CPPD, t-CPPD, m-CPPT β and m-CPPM) are first reported, followed by those of amorphous calcium pyrophosphates. Then, we report the results of first-principles calculations of the NMR parameters of the crystalline calcium pyrophosphate phases, which were carried out using the Gauge-Including Projector Augmented Wave (GIPAW) approach [37,38]. The comparison between experimental and calculated NMR parameters not only validates the structural models of each compound allowing the assignment of P and Ca sites in crystalline phases, but also helps determine what atomic-scale information can be determined by solid state NMR. Interpretations of the NMR spectra of amorphous calcium pyrophosphate are given, a phase that has recently been proposed as an interesting component of bone cements [39].

2. Materials and methods

2.1. Syntheses

Crystalline hydrated calcium pyrophosphates m-CPPD, t-CPPD and m-CPPT β were synthesized following the methods previously published by Gras [10], by double decomposition between a potassium pyrophosphate solution and a calcium nitrate solution mixed into a buffer solution at a controlled temperature. The crystalline m-CPPM phase was prepared starting from m-CPPT β crystals and heating them at 110 °C for 30 min as previously reported by Gras et al. [12].

The amorphous calcium pyrophosphate phases, of general formula $Ca_2P_2O_7.xH_2O$ (with $x \sim 4$) [10,13], were prepared using two different synthetic procedures. According to Gras [10], precipitation at a controlled temperature (25 °C) and pH (5.8) was used (compound referred to as "sample A" thereafter). According to Slater et al. [13] a precipitation at room temperature without any specific control/monitoring of the pH was also performed (compound referred to as "sample B" thereafter). In the latter case, the amorphous phase was also heat treated to 140 and 220 °C, in view of further solid state NMR characterizations of the transformations under temperature.

2.2. Characterization

2.2.1. General characterization

XRD measurements were performed using a Seifert XRD-3000TT diffractometer with a Cu Kα radiation (Cu $K\alpha_1$ λ = 1.54060 Å and Cu $K\alpha_2$ λ = 1.54443 Å), and equipped with a graphite monochromator. The XRD patterns were obtained between 2° and 70° (2θ) with a step size of 0.02° and a scan step time of 16 s at 298 K. The corresponding XRD powder patterns can be found in supporting information (Figure S1). The other characterization performed on the crystalline and amorphous synthesized phases, using notably vibrational spectroscopies, can be found in previous publications [10,13].

2.2.2. ^{31}P solid state NMR

All ^{31}P MAS NMR data were recorded at 14.1 T using a VNMRS-600 (600 MHz ^{1}H frequency) spectrometer operating at a ^{31}P Larmor frequency of 242.81 MHz, using a Varian 3.2 mm HXY T3 MAS probe. MAS frequencies ranging from ~2.8 to 10 kHz were used in order to extract the chemical shift anisotropy (CSA) parameters. Experiments were performed using a set of saturation ^{31}P pulses, followed by a delay of 128 s, and a 90° excitation pulse of 2.5 μs. Spinal-64 ^{1}H decoupling (100 kHz RF) was applied during acquisition [40]. A total of 4 transients were acquired for each spectrum. The ^{31}P chemical shifts were referenced to $Si_5O(PO_4)_6$ as a