NMR spectroscopic study of organic phosphate esters coprecipitated with calcite

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Abstract

Organic phosphorus incorporated in calcite during laboratory precipitation experiments and in natural cave deposits was investigated by solid-state NMR spectroscopy. For calcite precipitated in the presence of organic phosphoesters of varying size and functionality, solid-state $^{31}P\{^1H\}$ CP/MAS NMR shows that the phosphoesters were incorporated intact into the solid. Systematic changes in the ^{31}P NMR chemical shift of the phosphate group were observed between the solid phosphoester and that incorporated in the solid precipitate, yielding ^{31}P NMR chemical shifts of the coprecipitates in the range of +1.8 to −2.2 ppm. These chemical shifts are distinct from that of similarly prepared calcite coprecipitated with inorganic phosphate, 3.5 ppm. Only minor changes were noted in the phosphoester ^{31}P chemical shift anisotropy (CSA) which suggests no significant change in the local structure of the phosphate group, which is dominated by C–O–P bonding. Close spatial proximity of the organic phosphate group to calcite structural components was revealed by $^{31}P/^{13}C$ rotational echo double resonance (REDOR) experiments for coprecipitates prepared with ^{13}C-labeled carbonate. All coprecipitates showed significant ^{31}P dephasing effects upon ^{13}C-irradiation, signaling atomic-scale proximity to carbonate carbon. The dephasing rate for smaller organophosphate molecules is similar to that observed for inorganic phosphate, whereas much slower dephasing was observed for larger molecules having long and/or bulky side-chains. This result suggests that small organic molecules can be tightly enclosed within the calcite structure, whereas significant structural disruption required to accommodate the larger organic molecules leads to longer phosphate–carbonate distances. Comparison of ^{31}P NMR spectroscopic data from the synthetic coprecipitates with those from calcite moonmilk speleothems indicates that phosphorus occurs mainly as inorganic orthophosphate in the natural deposits, although small signals occur with characteristics consistent with phosphate monoesters. The results of this study indicate that trace- to minor concentrations of dissolved organic molecules can be effectively taken up during calcite precipitation and incorporated in the structure, leaving a resilient record of materials present during crystallization.
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1. INTRODUCTION

As potential recorders of paleoenvironmental information and natural laboratories for understanding control of impurity and trace element distribution during crystallization, calcium carbonate minerals such as calcite continue to be widely studied. During calcite crystal growth, many

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components of the aqueous fluid adsorb to the mineral surface and can be entrapped or incorporated into the mineral at trace to minor concentrations (Watson, 2004) in such a way as to potentially provide a record of the environmental conditions and solution composition at the time of crystallization (e.g., Borsato et al., 2007; Blythe et al., 2008; Perrette et al., 2005; Fairchild and Treble, 2009). Among the impurities in calcite precipitated in low-temperature environments are large organic molecules, such as humic and fulvic acids (Neuweiler et al., 2000; Smailer and White, 2013), lipids (Pearson et al., 2005; Blyth et al., 2011), and amino acids (Walton, 1998; Penkman et al., 2008), that cannot be incorporated in calcite by simple substitution. Such organic molecules could be useful for extracting paleoenvironmental information if they are incorporated in the calcite crystals at the time of deposition and remain impervious to removal or replacement over geological timescales. Release of organic molecules by digestion of calcite after prolonged exposure to strongly oxidizing solutions is generally accepted as evidence for the presence of intracrystalline organic matter in calcite from biominerals (Berman et al., 1990; Albeck et al., 1993; Aizenberg et al., 1994; Marin et al., 2005; Pokroy et al., 2006a; Nudelman et al., 2007; Okumura et al., 2013) and of abiotic origin (Lauritzen et al., 1994; Sykes et al., 1995; Rousseau et al., 1995; Neuweiler et al., 1999, 2000; Pearson et al., 2005; Huang et al., 2008). For example, from SEM observations Ramseyer et al. (1997) concluded that during abiotic precipitation organic molecules adsorbed to the mineral surface are incorporated into the mineral structure during crystal growth. Such "intracrystalline" organic matter could be a faithful recorder of crystallization conditions if it can be ascertained that the organic molecules do indeed occupy structural positions in the mineral.

Owing to the conformational and size mismatch between such organic molecules and calcite structural components, the relationship between occluded or intracrystalline organic matter and the calcite structure is not clear. An understanding of this structural relationship is crucial for evaluating the probability that impurities of interest were deposited coevally with the calcite and not altered by subsequent external processes. Occurrence as large structural defects would offer promise that the impurities have been preserved since the time of crystallization and that some information about the composition of the fluid can be inferred from abundances and nature of organic molecules and other trace elements adsorbed to them (Borsato et al., 2007; Fairchild and Treble, 2009). On the other hand, occurrence primarily as inclusions, particulate matter, or as intragranular coatings would offer more challenging prospects of developing robust interpretations from ancient deposits. Distinguishing among these (and potentially other, unimagined) possibilities remains a difficult problem because of the typically low concentrations, heterogeneous nature of the organic material and sub-microscopic scale of the relevant features.

Among the few experimental techniques available to investigate at the atomic scale the relationship between calcite structural components and occluded organic molecules,

NMR spectroscopy can provide information on elementally specific spatial proximities in a manner helpful for distinguishing structural components from inclusions and other potential locations of organic matter. In addition to observation of NMR parameters, such as chemical shift, to fingerprint chemical or structural environments, more complex NMR double resonance methods enable interatomic distances to be sensed through measurement of dipolar couplings. The magnetic dipole–dipole interaction between NMR-active nuclei varies with the inverse-cube of the internuclear distance, making it effective for detecting atomic-scale spatial relationships among atoms separated by distances of less than about 5 Å. However, investigation of organic matter in calcite with such methods is challenging because the predominant naturally occurring isotopes of calcite (^{40}Ca, ^{12}C, ^{16}O) are NMR-inactive, the typical concentrations of organic matter approach the practical detection limit for NMR, and the most easily accessed NMR-active nuclide in calcite, ^{13}C, also occurs in the organic molecules. In addition, exploiting potentially useful heteronuclear interactions between ^1H of organic molecules and carbonate carbon is complicated by the common occurrence of structural water and other forms of H in calcite as an additional source of ^1H signal (Gaffey, 1995; Feng et al., 2006). In a previous study, Phillips et al. (2005) circumvented this problem by preparing citrate/calcite coprecipitates in D_2O solutions, leaving the occluded citrate as the dominant source of ^1H signal. In this case it was possible to detect the presence of the citrate methylene H near carbonate carbon atoms, which had been enriched in ^{13}C in the synthesis, showing that the citrate molecules occur in the calcite structure.

Another potential NMR-based experimental approach is to investigate the proximity of carbonate carbon to other components of organic molecules such as nitrogen and phosphorus (P), using $^{13}C/^{14}N,^{15}N$ or $^{13}C/^{31}P$ NMR double resonance techniques. For example, in an investigation of the amorphous calcium carbonate (ACC) component of crayfish gastroliths, Akiva-Tal et al. (2011) observed ^{13}C resonances for chitin as well as for small metabolites such as citrate and phosphoenolpyruvate (PEP). The signals for these organic molecules were not present in spectra taken after demineralization, suggesting that they occur in the ACC. In $^{13}C/^{31}P$ double resonance experiments (heteronuclear correlation), clear cross-peaks were observed between the ACC carbonate ^{13}C peak and the ^{31}P signals for inorganic phosphate and PEP. This result indicates that both inorganic phosphate and PEP occur in the ACC structure with atomic-scale proximity to carbonate groups. A similar approach was taken by Gertman et al. (2008) to study the nature of impurities in calcite coccoliths. In this case, algae were grown in a medium isotopically enriched in ^{13}C and ^{15}N, which enabled application of NMR double resonance experiments to explore the structural relationship among components of the calcite biomineral. Although a variety ^{15}N and ^{31}P resonances were apparent in spectra of the coccoliths, including several from organic components, these signals were much reduced or absent from samples treated in NaOCl solutions. This result implied that the signals from