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A refined protocol for $\delta^{18}O_{PO4}$ analysis of conodont bioapatite

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ABSTRACT

The isotopic composition of phosphate-bound oxygen ($\delta^{18}O_{PO4}$) in bioapatite ($Ca_{10}(PO_4,CO_3)_6(OH,F,Cl)_2$) is increasingly used in paleoclimatology, paleoecology, and paleoceanography studies as a proxy for environmental conditions during mineral precipitation. However, a minimum total sample size (≥0.5 mg to >1 mg) required for requisite triplicate analysis can limit the spatial and temporal application of the proxy, in particular in studies using microfossils (e.g., conodonts) for $\delta^{18}O_{PO4}$ analysis. Here we present a systematically evaluated extraction-conversion-analysis protocol for conodont $\delta^{18}O_{PO4}$ of total sample mass as small as 0.3 mg while maintaining standard analytical precision (~0.3‰, 1σ). These results were produced by systematic testing of existing methodologies in combination with the vario PYRO cube high-temperature pyrolysis system. Our data indicate that bulk carbonate samples dissolved in 7% acetic acid buffered to a pH of 3.6 provide the highest conodont yields while minimizing bioapatite etching, thus avoiding potential oxygen isotope fractionation due to selective dissolution of conodont material. Isolating phosphate from the other components of dissolved bioapatite using a highly buffered silver amine solution produces larger silver phosphate crystals and greater phosphate yields compared to the standard silver amine protocol. Analysis of silver phosphate using a high-temperature pyrolysis system with reverse helium flushing and a thermodynamic carbon monoxide trap generates reproducible $\delta^{18}O$ values (0.1 to ≤0.4‰, 1σ) on atypically small bioapatite samples (0.3 mg). Application of this refined technique to Middle Pennsylvanian age conodonts from Arrow Canyon, NV, demonstrates the potential of the conodont $\delta^{18}O_{PO4}$ proxy for high temporal resolution (sub-10^4-yr) studies. Further, we propose a $\delta^{18}O_{PO4}$ methodology to serve as a community standard that will permit more effective inter-dataset comparisons by limiting the potential influence of methodological variability on $\delta^{18}O_{PO4}$ values.

1. Introduction

The skeletal components of many marine and terrestrial organisms occur as the mineral bioapatite ($Ca_{10}(PO_4,CO_3)_6(OH,F,Cl)_2$), providing the opportunity to use the $\delta^{18}O$ of phosphate-bound oxygen ($\delta^{18}O_{PO4}$) as a proxy for oceanographic, ecological, and anthropological studies (Tudge, 1960; Longinelli and Nuti, 1973; Wright and Hoering, 1989; Kohn and Cerling, 2002; Lécuyer et al., 2003). $\delta^{18}O_{PO4}$ has been used as a tracer of phosphate cycling in both terrestrial (Tamburini et al., 2010; Angert et al., 2012) and marine environments (Jaisi and Blake, 2010; Goldhammer et al., 2011); to infer thermoregulation (Barrick and Showers, 1994; Bernard et al., 2010); as well as to track the behavior, migration, and origin of organisms, including humans (Gehler et al., 2012; Chereny et al., 2012). It has long been argued that bioapatite $\delta^{18}O_{PO4}$ is a more robust proxy of surface conditions during mineralization than other oxygen-bearing ions, including the more commonly analyzed carbonate component of biological apatite and calcium carbonate, given phosphate's lower susceptibility to diagenetic alteration (Longinelli and Nuti, 1973; Lécuyer et al., 1999; Kohn and Cerling, 2002). Experimental results demonstrate that carbonate-bound oxygen exchanges with the oxygen in post-depositional fluids at a rate ten times that of phosphate-bound oxygen (Zazzo et al., 2004), reflecting the greater strength of the phosphorus-oxygen bond (Sponheimer and Lee-Thorp, 1999).

Despite the potential use of fossil $\delta^{18}O_{PO4}$ as a robust proxy of paleotemperature (Pucéat et al., 2010; Lécuyer et al., 2013) and paleohydrologic conditions (Fricke et al., 1998; Suarez et al., 2012), its utility can be hampered by sample size. Conventional external precision of $\delta^{18}O_{PO4}$ measurements is ~0.3‰ (1σ), which requires triplicate analysis of samples of bioapatite that in turn requires a total sample mass of ~0.5 to >1 mg (Joachimski et al., 2009, 2012; Theiling et al., 2012; Rosenau et al., 2014). Higher precision is typically obtained by processing larger samples (Chereny et al., 2012). This sample size requirement thus limits the spatial and temporal resolution at which bioapatite-based proxy records can be developed. LaPorte et al. (2009) proposed a combination of cation exchange and $\delta^{18}O_{PO4}$ analysis with a back-flushing pyrolysis system for unusually small bioapatite samples (0.2 mg) with

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unprecedented external precision (±0.15‰, 1σ). To date, this method has not been widely adopted given the expensive and labor-intensive nature of the chemistry. As such, the requisite sample size for $\delta^{18}O_{PO4}$ analysis, in particular for conodonts recovered from marine limestones characterized by low fossil yields, can constrain the sampling resolution of stratigraphic successions and the nature of the scientific questions that can be addressed using this proxy. An efficient, cost-effective alternative to past approaches is needed.

Increasingly, the $\delta^{18}O_{PO4}$ of conodonts is being used to reconstruct Paleozoic and early Mesozoic seawater oxygen isotopic composition ($\delta^{18}O_{SW}$), which in turn is used to estimate variations in ice volume and glacioeustasy during icehouses (Joachimski et al., 2006; Elrick et al., 2009; Elrick and Scott, 2010; Theiling et al., 2012) or the evolution of sea surface temperatures in paleoceans (Joachimski et al., 2012; Sun et al., 2012; Chen et al., 2013). Conodont microfossils are the mineralized remains of an extinct, nektonic, soft-bodied marine animal that can be found in Cambrian through Triassic shales and limestones (Sweet and Donoghue, 2001). Although abundant in some shales, conodont occurrences in limestones range from more than 100 conodonts to as low as a few elements per kilogram (Fohrer et al., 2007; Griffin and Montañez, 2013). Furthermore, conodont mass is highly variable due to the many different types of elements, so sample sizes ranging from ≤10 to hundreds of conodont elements are required to attain 1 mg of bioapatite (e.g., Rigo and Joachimski, 2010). Conodont elements of different genera potentially mineralized in different portions of the water column (Seddon and Sweet, 1971; Joachimski et al., 2006, 2012; Herrmann et al., 2010; Sun et al., 2012; Chen et al., 2013), thus amalgamation of the elements can homogenize $\delta^{18}O_{PO4}$ variability. Given typical conodont yields in limestones, it is common that several kg of limestone must be processed in order to provide adequate bioapatite mass for $\delta^{18}O_{PO4}$ analysis; with sampling constraints and long-term accumulation rates of a few cm/kyr or less, sampling resolution is limited to the 10^4- to 10^6-yr scale (MacLeod, 2012). Many studies have documented that climate perturbation through solar, orbital, and greenhouse-gas forcing can occur at timescales well below this resolution and can be archived in the deep-time geologic record (Montañez et al., 2011). Applying conodont $\delta^{18}O_{PO4}$ to evaluate environmental change at the ≤10^4-yr-scale requires the ability to provide reproducible bioapatite measurements from samples considerably smaller than typically employed.

Here we present a comprehensive extraction-conversion-analysis protocol for obtaining conodont $\delta^{18}O_{PO4}$ values on samples of a total size as small as 0.3 mg with an analytical precision of 0.1 to ≤0.4‰ (1σ). The protocol is based on systematic testing of existing methodologies with the goal of maximizing conodont yields (conodonts per kilogram of rock) from limestone samples while minimizing conodont leaching and any possible isotopic fractionation as an artifact of the method, as well as maximizing yields from the conversion of bioapatite to silver phosphate. The combination of this protocol and a new pyrolysis system, the vario PYRO cube, which employs a molecular trap and back-flushing He flow system, reduces the amount of silver phosphate necessary for accurate and precise $\delta^{18}O_{PO4}$ analyses. While the methods for extraction and chemical conversion discussed in this paper are mostly published and recently reviewed (MacLeod, 2012), this study is the first to systematically test existing outcrop-to-$\delta^{18}O_{PO4}$ methodologies and then apply the results to define a best-practice protocol for conodont $\delta^{18}O_{PO4}$ studies in deep-time.

2. Methodology

The many variations in the protocol for producing conodont $\delta^{18}O_{PO4}$ values will be reviewed in the following sections. In general, conodonts are extracted from limestone by acid dissolution, followed by heavy liquid separation of the residue and handpicking of the heavy fraction under a binocular scope (Harris and Sweet, 1989). Phosphate is isolated from the conodonts as silver phosphate (Wright and Hoering, 1989), which is converted to CO gas by pyrolysis and analyzed for $\delta^{18}O$ by continuous-flow isotope ratio mass spectrometry (Vennemen et al., 2002; Lécuyer et al., 2007). The conodonts analyzed for $\delta^{18}O_{PO4}$ in this study were extracted from limestone samples (~5 kg each) collected from the Middle Pennsylvanian (Desmoinesian) part of the Bird Spring Formation in Arrow Canyon, Nevada (N 36.73002, W 114.77000) using the stratigraphic framework of Cecil et al. (2003) and Bishop et al. (2010). Conodont alteration index (CAI) values for the conodonts are ≤1.5 (Epstein et al., 1977), so thermal alteration of $\delta^{18}O_{PO4}$ during burial is minimal (Wheeley et al., 2012).

2.1. Conodont extraction from limestones

Conodonts are extracted from limestones using varying concentrations of formic (Jeppsson et al., 1999) and glacial acetic acid (Harris and Sweet, 1989), despite documentation that some acids can dissolve conodonts, thus decreasing conodont yield and potentially introducing artifacts via selective dissolution. For example, prolonged exposure to formic acid has been shown to etch conodonts (Cooper and Whittington, 1965), and conodonts dissolve overnight in low concentrations of glacial acetic acid (5%) (Jeppsson et al., 1985). Moreover, conodonts extracted with 10% formic acid can deviate in $\delta^{18}O_{PO4}$ by as much as 1.8‰ from those extracted with 10% glacial acetic acid (Wheeley et al., 2012). Jeppsson et al. (1999) proposed using 7% acetic acid buffered with calcium acetate to a pH of 3.6 in order to prevent selective dissolution of microfossil bioapatite during extraction. Despite documentation that buffered acetic acid dissolution results in the highest conodont yields (Mawson, 1987), this approach is rarely employed in conodont $\delta^{18}O_{PO4}$ studies.

To investigate this further and maximize conodont yield from limestones, we carried out a series of experiments using glacial acetic acid (GAA). Three aliquots of one sample were split such that two were dissolved in 7% and 10% GAA, the concentrations typically used in conodont $\delta^{18}O_{PO4}$ studies, and one was dissolved in 7% GAA buffered to a pH of 3.6 (method of Jeppsson et al., 1999). When the solution reached a pH of 5 (usually after four days), samples were wet sieved using nested 2 mm and 0.063 mm sieves (Stone, 1987), and the fractions >2 mm were re-immersed in fresh batches of their respective solutions. This procedure of dissolution, sieving, and re-immersion was carried out for 20 days. Compiled, dried sand-sized residues were separated using lithium polytungstate (LST) heavy liquid (Krukowski, 1988) and handpicked under a binocular microscope to determine initial conodont yield. Sample fractions >2 mm were re-immersed in their respective solutions until complete limestone dissolution occurred. Extracted conodonts were imaged using a Hitachi TM 3000 Tabletop eSEM in order to evaluate evidence of corrosion.

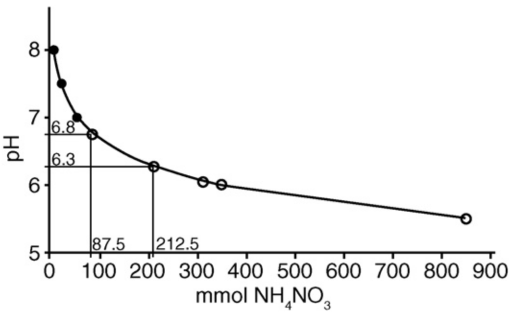

Fig. 1. Relationship between mmoles NH_4NO_3 in silver amine solution and pH during phosphate precipitation plotted using the data of Firsching (1961) (closed circles). The logarithmic relationship ($y = -0.549*\ln(x) + 9.2258$, $R^2 = 0.99$) was extended to include silver amine solutions with higher NH_4NO_3 concentrations (open circles) in order to calculate at what pH all of the soluble PO_4^{3-} is anticipated to precipitate as Ag_3PO_4 crystals. Tie lines indicate the pH relationship for the range of solution NH_4NO_3 concentrations used in this study.