Oxygen isotope signature during phosphite oxidation by bacterial alkaline phosphatase

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Phosphorus (P) is commonly considered to be a redox conservative element in living organisms and most P found in biological intermediates, including inorganic phosphate and organic phosphate esters, is fully oxidized (+5 valence state). However, it is increasingly apparent that many organisms are capable of metabolizing reduced P compounds, indicating that biological P redox reactions possible and even likely. Recent discoveries have shown that *Escherichia coli* bacterial alkaline phosphatase (BAP), a most extensively studied enzyme for its phosphomonoesterase activity, can also catalyse the oxidation of phosphite (PO_3 with P^{3+}) to phosphate (PO_4 with P^{5+}) [1]. We investigated the mechanisms of oxygen transfers and O-isotopic fractionation in reactions of cell-free BAP-catalyzed PO_3 oxidations using the technique of multi-labeled water isotope probing (MLWIP).

The BAP-catalyzed oxidation of phosphite was conducted using ^{18}O-labeled phosphite and waters at pH 7 and 37 °C. Aqueous phosphite (13-19%) was very slowly oxidized to phosphate over 1 year. The $\delta^{18}O$ values of product phosphates varied with the $\delta^{18}O$ values of ambient waters. Slopes of linear regressions of $\delta^{18}O_{PO4}$ vs. $\delta^{18}O_{H2O}$ data were ~0.8, which indicates that ~80% of oxygen in product phosphate was derived from ambient water. Additionally, ^{18}O is preferentially incorporated into product PO_4 with a fractionation of +26 ‰. These results suggest that the BAP-catalyzed oxidation of PO_3 involves at least a 2-step pathway: oxidation of PO_3 to PO_4 (Step 1) and, subsequent O-isotope exchange between PO_4 and water (Step 2). These results have broad implications, most notably with respect to the biochemistry of P redox reactions, but also with respect to the interpretation of PO_4 $\delta^{18}O$ bio-signatures preserved in ancient terrestrial and extraterrestrial samples, such as from Mars where reduced P compounds might be abundant as prebiotic P sources under an oxygen-free environment.

[1] Yang & Metcalf (2004) *PNAS* 101, 7919.
[2] Herschy et al. (2018) *Nature Communications* 9, 1346.