Metabolic pathways of anaerobic phosphite oxidation in *Desulfotignum phosphitoxidans*

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A sulfate-reducing bacterium, *Desulfotignum phosphitoxidans*, that uses phosphite (PO_3, +3 valence) as an electron donor for anaerobic growth has been discovered from marine sediments [1]. Phosphite dehydrogenase (PtxD), a unique enzyme that catalyses the oxidation of PO_3 to phosphate (PO_4, +5 valence) with concurrent reduction of NAD^+ to NADH [2], was identified in *D. phosphitoxidans* by genetic and biochemical studies [3]. We investigated the mechanisms of oxygen transfers and O-isotopic fractionation in reactions of: 1) cell-free PtxD-catalyzed PO_3 oxidations, and 2) microbial metabolism of PO_3 by *D. phosphitoxidans,* using the technique of multi-labled water isotope probing (MLWIP).

During PtxD-catalyzed oxidation of PO_3 under aerobic conditions, one oxygen atom in product PO_4 is derived from ambient water without O-isotopic fractionation and all 3 oxygens in the original PO_3 are inherited in product PO_4. Microbial metabolism of PO_3 by intact, whole cells of *D. phosphitoxidans*, involves the additional action of inorganic pyrophosphatase (PPase), which catalyzes complete, temperature-dependent, equilibrium exchange of all 4 oxygen atoms in PO_4 with ambient water. These combined results suggest that the microbial metabolism of PO_3 involves at least a 2-step intracellular enzyme-catalyzed pathway: PtxD-catalyzed oxidation of PO_3 to PO_4 (Step 1) and, subsequent PPase-catalyzed O-isotope exchange between PO_4 and intracellular water (Step 2) [4]. Moreover, PO_4 produced by *D. phosphitoxidans* does not show a metabolic water signature, suggesting that O_2 is the key factor in producing the metabolic water signature observed previously in aerobic microbes [5]. These results will advance interpretations of PO_4 $\delta^{18}O$ biosignatures preserved in ancient terrestrial and extraterrestrial samples.

[1] Schink & Friedrich (2000) *Nature* 406, 37.[2] Metcalf & Wolfe (1998) *J. Bacterial.* 180, 5547. [3] Simeonova et al. (2010) *J. Bacterial.* 192, 5237. [4] Chang & Blake (2010) *GCA* 150, 314. [5] Li et al. (2016) *PNAS* 113, 5862.