# Kinetics of oxygen isotope exchange between dissolved phosphate and water catalyzed by inorganic pyrophosphatase from 3-26 ºC

S.J. CHANG[1,2]*, R.E. BLAKE[2] AND A.S. COLMAN[3]

[1]Korea Basic Science Institute, Chungbuk 28119, Republic of Korea (*correspondence: sjchang15@kbsi.re.kr)

[2]Dept. of Geology & Geophysics, Yale University, New Haven, CT 06511, USA (ruth.blake@yale.edu)

[3]Dept. of the Geophyscial Sciences, The University of Chicago, Chicago, IL 60637, USA (asc25@uchicago.edu)

In natural aqueous systems, reactions involving *ortho*phosphate (represented here by $PO_4$ to encompass the range of protonation states and ion pairs) are primarily carried out by microorganisms and catalyzed by enzymes. The O-isotope composition ($\delta^{18}O$) of $PO_4$ is a widely used (paleo)thermometer [1], biomarker [2] and useful tracers of P biogeochemical cycling [*e.g.*, 3] and intracellular reactions [4]. Recent evidence points to inorganic pyrophosphatase (PPase) as the key enzyme responsible for both the equilibrium and temperature dependence of dissolved $PO_4$-$H_2O$ O-isotope exchange [5]. Calibration of equilibrium O-isotope fractionations between $PO_4$ and $H_2O$, catalyzed by PPase, was experimentally determined from 3 to 37 ºC [6].

Here, we present experimentally-determined kinetics of O-isotope exchange between dissolved $PO_4$ and $H_2O$, catalyzed by PPase, from 3-26 ºC. O-isotope exchange reactions were conducted using $^{18}O$-labeled $PO_4$ and waters in the presence of PPase (0.16 units/μmole $PO_4$) for a week in buffered solution at pH 7.4. The data are well described by first order reaction kinetics (rate constant $k$ = 9E-05 to 2E-04 sec$^{-1}$; $t_{1/2}$ = 64 to 696 min). The temperature dependence of the exchange reaction is well fit by the Arrhenius equation, and the activation energy is ca. 65-70 kJ/mole. The rate of PPase-catalyzed reaction is ca. 8 orders of magnitude faster than the rate of abiotic reaction (pH 5) at 20 ºC calculated by extrapolation of high temperature rate data [7]. Results from this study may be used to improve interpretation of measured $\delta^{18}O$ values of dissolved $PO_4$ in nature and cellular reactions (*e.g.*, distinction between microbial overall, PPase-catalyzed or other enzymatic rates of evolution of $\delta^{18}O$ values).

[1] Longinelli & Nuti (1973) *EPSL* **19**, 373-376. [2] Blake *et al.* (2001) *PNAS* **98**, 2148-2153. [3] Colman *et al.* (2005) *PNAS* **102**, 13023-13028. [4] Li *et al.* (2016) *PNAS* in review. [5] Blake *et al.* (2005) *Am. J. Sci.* **305**, 596-620. [6] Chang & Blake (2015) *GCA* **150**, 314-329. [7] Lecuyer *et al.* (1999) *GCA* **63**, 855-862.