Enzyme-catalyzed oxygen isotope exchange between inorganic phosphate and water: Reaction rates and temperature dependence at 5.7–30°C

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The resistance of phosphate minerals to isotopic exchange with water and diagenetic alteration makes the oxygen isotope composition of phosphates a very attractive palaeoenvironmental indicator as well as a potential conservative tracer of dissolved inorganic PO4 (Pi) as it is cycled through aquatic and sedimentary environments as a nutrient or pollutant. Currently used phosphate oxygen isotope thermometer equations were developed solely on the basis of empirical measurements of biogenic phosphates such as shells and teeth (e.g. Longinelli and Nuti, 1973; Lecuyer *et al.*, 1996), however, these relations have not been verified by laboratory controlled equilibrium isotope exchange experiments due to sluggish reaction rates at low temperature. It is widely accepted that the oxygen in phosphate is readily exchanged and equilibrated with body water of living organisms *via* multiple enzyme-catalyzed metabolic reactions. Here we report results of experiments conducted using purified cell-free enzymes, to facilitate oxygen isotope exchange between dissolved Pi and water under well-controlled conditions of temperature and oxygen isotope composition of water (δ^{18}Ow) with the aim of obtaining a laboratory calibration of the phosphate-water oxygen isotope thermometer at low temperature.

The most elementary system for Pi-water oxygen isotope exchange would consist of: orthophosphate + water + enzyme. The enzyme inorganic pyrophosphatase (Ppiase) is known to catalyze significant amounts of oxygen isotope exchange between Pi and water in this system during the overall reaction:

$$\underset{\text{PPi}}{H_2P_2O_7^{2-}} + H_2O \overset{Mg^{+2}}{\underset{\text{PPiase}}{\rightleftarrows}} \underset{\text{Pi}}{2HPO_4^{2-}} + 2H^+ \qquad (1)$$

(Cohn, 1958; Janson *et al.*, 1979) and, therefore, was used in our experiments to promote equilibrium oxygen isotope exchange. Inorganic pyrophosphatase is ubiquitous in the cells of living organisms where it catalyzes reaction (1) for pyrophosphate (PPi), released following the enzymatic hydrolysis of ATP to give AMP (adenosine monophosphate) + PPi, which helps to drive many energy-consuming cellular processes (e.g. Walsh, 1979). The use of cell-free, purified enzymes also offers the advantages of (1) limiting the variables and chemical species occurring in the reaction medium, (2) allowing specific reaction mechanisms to be probed, and (3) catalyzing these reactions in the absence of vital effects which may be inherent in living organisms.

Results of experiments conducted with waters having a wide range of δ^{18}Ow values demonstrate progressive oxygen isotope exchange from opposite sides of the equilibrium fractionation (Fig. 1). Isotopic fractionation factors ($10^3\ln\alpha$) measured in experiments run in waters with both high (14.2 to 99.0‰) and low (−19.7 to −19.50‰) δO values rapidly approached constant values in the presence of PPiase with over 90% exchange in 0.5 h at 30 °C. Enzyme assays performed over the entire course of experiments demonstrate that the approach of $10^3\ln\alpha$ to constant values was not due to a decrease in enzyme activity or deactivation of the enzyme.

The temperature dependence of the reaction catalyzed by PPiase was evaluated by comparing results of experiments run between 5.7 and 30°C for up to 125 h (Fig. 2). The temperature dependence of the fractionation of oxygen isotopes between dissolved phosphate and water calculated from these data is 0.15‰/°C, which is significantly lower than the value of 0.23‰/°C for biogenic apatite and water determined by Longinelli and Nuti (1973), but very close to the value of 0.16‰/°C determined for apatites synthesized from bacterially processed phosphates, reported by Blake *et al.* (1997). The difference between the apparent equilibrium fractionation relations for biogenic apatites *vs* enzyme and microbially processed phosphates may reflect

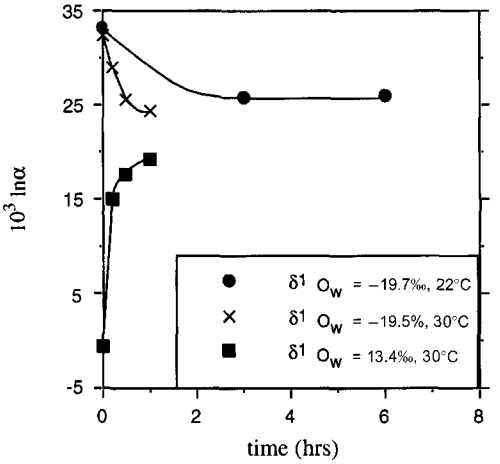

FIG. 1. Inorganic pyrophosphatase exchange experiments. Two-directional approach to equilibrium between dissolved inorganic phosphate (P_i) and water. Experiments carried out for 125 hours total.

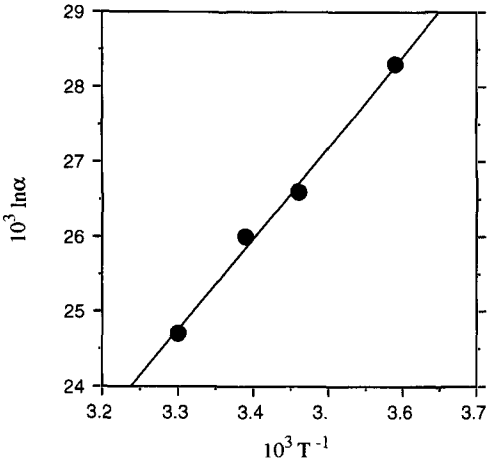

FIG. 2. Temperature dependence of oxygen isotope exchange catalyzed by PPiase between 5.7 and 30°C. $10^3 \ln\alpha = 12.2(10^3 T^{-1}) - 15.4$.

different metabolic pathways and isotope exchange reaction mechanisms. Enzyme-catalyzed isotope exchange reactions may be useful in the investigation of fractionation factors and reaction mechanisms in other systems at low temperature such as the sulphate/sulphide system.

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