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The effect of phosphomonoesterases on the oxygen isotope composition of phosphate

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Abstract

Plants and microorganisms under phosphorus (P) stress release extracellular phosphatases as a strategy to acquire inorganic phosphate (P_i). These enzymes catalyze the hydrolysis of phosphoesters leading to a release of P_i. During the enzymatic hydrolysis an isotopic fractionation (ε) occurs leaving an imprint on the oxygen isotope composition of the released P_i which might be used to trace phosphorus in the environment. Therefore, enzymatic assays with acid phosphatases from wheat germ and potato tuber and alkaline phosphatase from *Escherichia coli* were prepared in order to determine the oxygen isotope fractionation caused by these enzymes. Adenosine 5′ monophosphate and glycerol phosphate were used as substrates. The oxygen isotope fractionation caused by acid phosphatases is 20–30‰ smaller than for alkaline phosphatases, resulting in a difference of 5–7.5‰ in $\delta^{18}O$ of P_i depending on the enzyme. We attribute the enzyme dependence of the isotopic fractionation to distinct reaction mechanisms of the two types of phosphatases. The observed difference is large enough to distinguish between the two enzymatic processes in environmental samples. These findings show that the oxygen isotope composition of P_i can be used to trace different enzymatic processes, offering an analytical tool that might contribute to a better understanding of the P-cycle in the environment.

1. INTRODUCTION

Phosphorus (P) is an essential nutrient for all living organisms, because it is a component of many biomolecules such as DNA, RNA, ATP, or phospholipids (Westheimer, 1987). In terrestrial systems, plants and soil microorganisms take up P as orthophosphate (P_i) from the soil solution. Very often, however, low P_i concentrations limit biological growth (Ehlers et al., 2010; Richardson et al., 2011). Among other strategies, some plants and microorganisms have developed the ability to exudate phosphatases to overcome P-deficient conditions (Zimmermann et al., 2003; Richardson et al., 2011). These enzymes catalyze the hydrolysis of organic phosphate esters releasing P_i. Extracellular phosphatases have been detected in marine systems (Perry, 1972; Hoppe, 2003), in freshwater lakes and rivers (Jansson et al., 1988; Chappell and Goulder, 1995), and in soils (Tadano and Sakai, 1991; Gilbert et al., 1999) and they might therefore have a significant influence on the phosphorus cycle. However, the extent to which these enzymes contribute to the availability of P_i in the environment is not yet clear (Gressel et al., 1996; Joner et al. 2000; Paytan and McLaughlin, 2007). The analysis of the oxygen isotope composition of P_i may contribute to a better understanding of P cycling processes (Frossard et al., 2011).

It has been shown that under ambient temperatures abiotic oxygen isotope exchange between phosphate and water is negligible (Johansen et al., 1989; O'Neil et al., 2003). In contrast, biological processes have significant effects on

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the oxygen isotope composition of P_i. Microbes preferentially take up lighter isotopologues of P_i leading to an accumulation of heavier isotopologues in the residual P_i (Blake et al., 2005). After uptake, P is cycled inside the cell in various biochemical processes (Westheimer, 1987). The ubiquitous intracellular enzyme pyrophosphatase catalyzes a complete oxygen exchange between enzyme-bound P_i and water, which leads to a thermodynamic isotopic fractionation (Cohn, 1958; Longinelli and Nuti, 1973; Blake et al., 2005). Laboratory and field experiments have shown that the oxygen isotope composition of P_i can be rapidly altered by living organisms (Paytan et al., 2002). Hence, once a phosphate molecule has entered a living cell, its oxygen isotope composition is quickly equilibrated with cell water. After cell death and cell lysis, equilibrated P_i molecules are released into the environment. In some environmental systems, P_i released from dead cells might dominate the oxygen isotopic composition of P_i (Colman et al., 2005; Tamburini et al., 2012). P_i released from organic compounds by extracellular phosphatases might have different oxygen isotope compositions compared to P_i which is in equilibrium with cell water (Liang and Blake, 2006, 2009). In order to use the oxygen isotope composition of P_i as a tracer in the environment, it is necessary to understand the effect of extracellular phosphatases on the oxygen isotope composition of released P_i.

Cohn (1949) was the first to show that during the enzymatic hydrolysis of phosphomonoesters one oxygen atom from the surrounding water is incorporated into the newly formed P_i. During the incorporation, an isotopic fractionation (ε) occurs. Liang and Blake (2006, 2009) determined the oxygen isotope fractionations caused by phosphomonoesterases, namely alkaline phosphatase and 5′ nucleotidase, and phosphodiesterases. While alkaline phosphatases are important in the marine P-cycle and in alkaline soils, in acidic environments, as in many soils, acid phosphatases are predominant (Eivazi and Tabatabai, 1977; Chen et al., 2002). Our aim was, therefore, to determine the effect of acid phosphatases on the oxygen isotope composition of released P_i. Furthermore, we wanted to compare the effects of different phosphomonoesterases with the aim to understand the mechanisms controlling isotopic fractionations. Our observations indicate that the oxygen isotope fractionation caused by acid phosphatases is 20–30‰ smaller than that caused by alkaline phosphatases. We hypothesize that different isotopic fractionations associated to different phosphomonoesterases are due to differences in the reaction mechanisms of the enzymes.

2. ENZYME MECHANISMS

Before discussing the results of our enzymatic assays, we briefly review the current models of reaction mechanisms for alkaline and acid phosphatases, because these are fundamental to understand the oxygen isotope fractionation. During the hydrolysis of phosphomonoesters by some phosphatases, a phosphoenzyme intermediate is formed, which, in a subsequent step, is hydrolyzed again producing free enzyme, alcohol and P_i (Fig. 1) (Hengge, 2005). The hydrolysis of the phosphoenzyme intermediate leads to

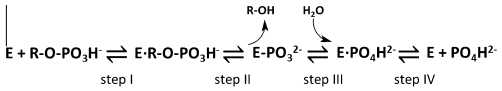

Fig. 1. Scheme of the reaction steps in the hydrolysis of a phosphomonoester by a phosphomonoesterase (Hengge, 2005).

the incorporation of one oxygen atom from water into the newly formed P_i molecule while three oxygen atoms are inherited from the original organic compound. The general scheme is valid for both alkaline and acid phosphatases, but there are some significant differences in the mechanism of reaction, which are key to interpret the observed oxygen isotope fractionations. In the following, we discuss these differences in more detail.

2.1. Reaction mechanism of alkaline phosphatase

Based on X-ray crystallographic studies, the following reaction mechanism for alkaline phosphatases from *Escherichia coli* has been proposed (Kim and Wyckoff, 1991; Stec et al., 2000). Catalysis in alkaline phosphatases is performed by two Zn^{2+} ions and a nucleophilic serine side chain. Furthermore, alkaline phosphatases contain a Mg^{2+} ion, but its role in catalysis is not entirely clear (Zalatan et al., 2008; López-Canut et al., 2011). The substrate is coordinated by the two Zn^{2+} ions and an arginine side chain (Fig. 2a). One of the two Zn^{2+} ions (Zn2) coordinates the hydroxyl group of the serine side chain, which is located opposite of the leaving group. Once the substrate is bound (step I), the serine acts as a nucleophile and attacks the phosphorus, which results in the formation of a covalent enzyme–phosphate intermediate and the release of the leaving group (Fig. 2a, step II). After dissociation of the leaving group, a hydroxide ion from the surrounding water takes the coordination site at Zn1 and displaces the phosphorus group from the serine, forming the enzyme–phosphate complex (Fig. 2a, step III). Subsequently, free orthophosphate is released to the medium (step IV). According to this mechanism, in the case of alkaline phosphatase, the oxygen atom attached to the phosphate is derived from a hydroxide ion.

2.2. Reaction mechanism of acid phosphatase

In the case of acid phosphatases, the active site consists of a nucleophilic histidine, four positively charged amino acid residues, namely three arginines and one histidine, and one acidic aspartate (Fig. 2b) (Lindqvist et al., 1994; Ortlund et al., 2003). The positively charged residues have two functions: on the one hand, they bind and orient the phosphate group of the phosphomonester, on the other hand, their positive charges depress the pK_a of the nucleophilic histidine. This shift in pK_a is the reason why the histidine is not protonated, even at low pH, and can therefore act as a nucleophile. Nucleophilic attack of the histidine on the phosphorus leads to formation of a covalent enzyme–phosphate intermediate and release of the leaving group (Fig. 2b, step II). Subsequently, the carboxylate of the aspartate deprotonates a water molecule in concert with the nucleophilic attack, which displaces the phosphoryl