PHYSICO-CHEMICAL PROPERTIES OF ALKALINE PHOSPHATASES RELEASED BY A PLANKTONIC CRUSTACEAN, DAPHNIA MAGNA (CLADOCERA)

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ABSTRACT

Our studies investigated the physico-chemical properties of alkaline phosphatase excreted by D. magna. This cladoceran mainly released alkaline phosphatase, though it also released a small amount of acid phosphatase. The alkaline phosphatase showed a broad pH optimum (8.05-10.0), and had a broad optimum temperature (30-35°C) with a temperature coefficient (Q10) of 2.45. The \( K_m \) of the enzyme is 0.15 ± 0.02 mM when p-nitrophenyl phosphate is used as a substrate, and the \( V_{\text{max}} \) is 0.43 ± 0.01 \( \mu \text{M pNP mg}^{-1} \text{DW h}^{-1} \). Even though alkaline phosphatase had been incubated in chloroform saturated with WC medium for 13 days, its activity was 54% that of the original. The enzyme was strongly inactivated by EDTA, and appeared to be zinc dependent. The alkaline phosphatase activity remained constant when D. magna was fed different quantities of Chlorella sp. The sensitivity of D. magna phosphatase activity to phosphate was time-dependent. During the first 16 hrs, the enzyme was insensitive to phosphate addition, after 24 hrs incubation the enzyme became sensitive to phosphate addition.

RÉSUMÉ

Nos études ont porté sur les propriétés physico-chimiques de la phosphatase alcaline excrétée par Daphnia magna. Ce cladocère libère principalement une phosphatase alcaline, bien qu’elle excrète aussi une petite quantité de phosphatase acide. La phosphatase alcaline présentait un large optimum de pH (8.05-10.0) et une température optimum étendue (30-35°C) avec un coefficient de température (Q10) de 2.45. Le \( K_m \) de l’enzyme est de 0.15 ± 0.02 mM quand le phosphate de p-nitrophényle est utilisé comme substrat, et le \( V_{\text{max}} \) est de 0.43 ± 0.01 \( \mu \text{M pNP mg}^{-1} \text{DW h}^{-1} \). Même lorsque la phosphatase alcaline était incubée dans du chloroforme saturé avec un milieu WC pendant 13 jours,
son activité était encore de 54% par rapport au premier test. L’enzyme était fortement inactivée par l’EDTA, et est apparue dépendante du zinc. L’activité de la phosphatase alcaline est restée constante, lorsque *D. magna* était nourrie avec des quantités différentes de *Clorella* sp. La sensibilité de l’activité de la phosphatase de *D. magna* par rapport au phosphate est fonction du temps : au cours des premières 16 heures, l’enzyme n’était pas sensible à l’addition de phosphate, mais après 24 heures d’incubation, elle y était devenue sensible.

INTRODUCTION

Freshwater eutrophication is caused by excessive nutrients, especially phosphorus (P), which is one of the most serious environmental problems. Phosphate is also the most commonly limiting nutrient in fresh waters. More than 90% of the total P in water is in organic form, present as living or dead particles (Wetzel, 2001). The actual availability of nutrients may determine the abundance of phytoplankton and the composition of the phytoplankton community. The major forms of phosphorus in which phytoplankton can take it up are dissolved inorganic phosphates, i.e., the ions $\text{H}_2\text{PO}_4^- + \text{HPO}_2^-$ (Cotner & Wetzel, 1991). Numerous publications have described alkaline phosphatase activity (APA) in aquatic ecosystems (Cembella et al., 1984; Jansson et al., 1988; Spijkerman & Coesel, 1998; Newman et al., 2003). Phosphatases, a group of enzymes that hydrolyse ester bonds between phosphates that are available for phytoplankton, are thus believed to play an essential role in the nutrient dynamics of lakes (Jansson et al., 1988), as well as in the bloom dynamics and the physiological ecology of the harmful algal bloom organism, *Proorocentrum minimum* (Pavillard Schiller), a dinoflagellate (Dyhrman, 2005). Phosphate is the main regulating factor for APA. When the concentration of soluble inorganic phosphate in the water is low, phosphatase will be produced by the phytoplankton (Cembella et al., 1984; Dyhrman, 2005), bacterioplankton (Cembella et al., 1984; Jansson et al., 1988; Neddermann & Nauch, 2004), and even by zooplankton (Rigler, 1961; Jansson, 1976; Wynne & Gophen, 1981; Boavida & Heath, 1984). This mechanism is especially critical when phosphate in the water is scarce.

Phosphatases have maximum catalysing activity at different pH values. Alkaline phosphatases (AP) have a pH optimum above 7, usually between pH 9 and 10, while the acid phosphatases’ highest expression is below pH 7, generally between pH 4 and 6 (Jansson et al., 1988). In addition, APA was shown to be sensitive to phosphate availability and particularly to the intracellular phosphate pool of microorganisms (Dyhrman, 2005). As a result, the APA is often used as an indicator for phosphate limitation (Healey, 1973; Gage & Gorham, 1985; Steinhart et al., 2002; Newman et al., 2003).

APA studies have mainly focused on algae (Healey, 1973; Giraudet et al., 1998; Spijkerman & Coesel, 1998; Nedoma et al., 2003), and have scarcely dealt with