EFFECT OF TEMPERATURE ON THE ACTIVITY OF ACID PHOSPHATASES ISOLATED FROM *CHLORELLA FUSCA, CLADOPHORA GLOMERATA* AND *PHORMIDIUM FAVEOLARUM* AND THERMAL STABILITY OF THE ENZYMES

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Abstract

Temperature effect has been investigated in this study on the phosphatases isolated from three algae representing different groups; a unicellular freshwater green alga *Chlorella fusca*, a filamentous green macroalga *Cladophora glomerata* and a filamentous freshwater blue green microalga, *Phormidium faveolorum.* The temperature dependence of the enzyme activity could be summarized as that 60 ºC was the optimum temperature for the enzyme activity in *Cladophora glomerata* as well as *Chlorella fusca* whereas *Phormidium faveolorum* had an optimum temperature of 70 ºC for both isolated enzyme fractions, the cell wall-bound and the secreted extracellular. The thermal behavior of the phosphatases isolated from the tested algae showed toleration to the thermal activation when p-nitrophenyl phosphate (pNPP) was utilized as a substrate which has been investigated to be itself is not sensitive to high temperatures. The two fractions of the enzyme isolated from the algae exhibited high stability at high temperatures even above 80 ºC

Key words: Acid phosphatase, Temperature, Thermal stability.

Introduction

The term phosphatases are commonly used for enzymes that catalyze the hydrolysis of esters and anhydrides of phosphoric acid (Feder, 1973). Phosphatases and kinases are responsible for the phosphorylation status of proteins in the cell. Kinases attach phosphoryl groups to a variety of compounds whereas phosphatases remove the phosphoryl group from protein, since in plant cells a variety of enzymes regulated by reversible phosphorylation have been identified (Huber *et al*., 1994).

Phosphatases and the factors affecting their activity were estimated to be primarily dependant on the type and concentrations of both the substrate and the enzyme. Other factors affecting phosphatase activity are ionic strength, metal ions, pH as well as temperature (McComb *et a*l., 1979) and some chemical compounds acting as enzyme inhibitors (Ibrahim *et al*., 2002; Ibrahim, 2005). Most enzymes have an optimum temperature, which may be related to the type of organism from which the enzyme was isolated. Some plants grow well near room

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temperature and so their enzymes are most active at a temperature around 30 to 40 ºC. Animal enzymes often have temperature optima near 37 ºC, especially human's (Jansson *et al*., 1988).

For thermophilic organisms like some bacteria and cyanobacteria that grow well in nearly boiling hot springs, much higher temperature optima were found, some enzymes even have temperature optima near 90 ºC (www. Bio. Intu. Edu / Campbell / b 1482).

Generally, phosphatases have temperature optima above the maximum temperature of natural waters, most often between 30 to 60 ºC. Huber and Kidby (1984) presented data from several investigations on natural waters and algal cultures showing phosphatase activity maxima mostly in the range 25 to 50 ºC. Some work has been done on the thermal stability of phosphatases and unusual optimum temperatures in plant enzyme. The purpose of this work is to examine the effect of the temperature on the activities of the phosphatases isolated from three species representing three different groups of algae, *Cladophora glomerata, Chlorella fusca and Phormidium faveolarum* trying to identify the optimum temperature for enzyme action and to study the thermal stability and thermal inactivation of the enzymes isolated from the selected species.

Materials and methods

Test organisms

Three algal species were chosen to perform tests on acid phosphatase activity. These species represent different algal groups; the unicellular green alga, *Chlorella fusca*; the filamentous branched green alga *Cladophora glomerata*; and the filamentous cyanophyte *Phormidium faveolarum.*

Chlorella fusca was obtained as a culture originating from the culture collection of Gottingen University, Germany. It was maintained in the laboratory on a selective solid medium (Grimme and Boardman 1972) for subsequent use. All the experiments performed for this alga were done in aerated liquid cultures. Filaments of *Cladophora glomerata* were collected from a freshwater stream in Minia town, Egypt. *Phormidium faveolarum* was isolated from a clay soil (Minia district), purified by streaking on agar plates and was kept on agar slants. Growth of the filamentous algae was maintained in Chu 10 liquid medium (Bold and Wynee 1978) and then filaments of the alga were collected by centrifugation, gently dried on filter paper and were stored at – 85 ºC for subsequent isolation.

Isolation of fractions enriched in bound and secreted acid phosphatases

Freezed tissues were used for preparing fractions of the cell wall -bound and the secreted acid phosphatases. About 0.6 g fresh weight of the alga were suspended in 5 ml Chu 10 medium, shaken gently and pelleted by centrifugation at 13,000 g for 15 min. at 4 ºC. The supernatant was collected and the algal pellets were resuspended in the same medium. Washing was repeated five times. The first and the second supernatants were pooled and represented the fraction of cell

wall-bound acid phospatase. The algal pellet was grown in the growing medium for 4 days at room temperature to obtain the secreted fraction of the enzyme. The secretion of the enzyme was followed according to Pfeiffer (1996).

Determination of optimum temperature and thermal stability of the enzyme

Experiments of determining the optimal temperature were performed in reaction mixtures buffered at pH 4.5 for *Cladophora,* 3.5 for *Chlorella* and pH 5.0 for *Phormidium*. Reaction mixtures were incubated for 45 min. at a temperature range from 20 to 90 ºC with 10 ºC intervals.

Experiments for determining the enzyme thermal stability were also performed in reaction mixtures buffered at the same pH values as the previous experiments of determining the optimal temperature. The enzyme containing fraction was preincubated in a reaction buffer without substrate (pNPP) for 45 min. at a temperature range from 20 to 90 ºC with 10 ºC intervals and then the enzyme activity was traditionally assayed with pNPP at 30 ºC for 45 min as reported by Pfeiffer (1996) by measuring the release of P-nitrophenol (pNP) from p- nitrophenyl phosphate (pNPP). Samples of 200 µl cotaining enzyme were incubated with 200 µl reaction buffer containing 40 mM Mes/Tris, pH 4.5, 5 mM pNPP and 10 mM MgCl2. 6 H2O for 45 min at 30 ºC. The reaction was stopped and the color was developed by addition of 800 µl of 400 mM borate buffer, pH 9.8. The concentration of pNP was determined using a linear regression of calibration standers. All experiments were performed in ice as triplicates.

Results and Discussion

The two fractions of the phosphatase enzyme isolated from *Cladophora glomerata, Chlorella fusca* and *phormidium faveolarum* presented high activities even at temperatures above 80 ºC (Fig. 1); recording an optimum temperature of 60 ºC for *Cladophora glomerata* and *Chlorella fusca* and 70 ºC for *Phormidium faveolarum*. These temperatures are higher than those described for other plant acid phosphatases such as for barley roots that ranged from 30 to 35 ºC (Panara *et a*l., 1990), for cotton seeds with 37 ºC optimum (Bhargava and Sacher 1987), for Yam with 50 ºC optimum (Kamenan and Dipoh, 1982), for cotyledons of germinating soybean seeds with 50 ºC optimum (Ullah and Gibson, 1988; Ferreira *et al.,* 1998), and for lily pollen grains with 37 ºC optimum (Ibrahim *et al*., 2002) when pNPP was utilized as a substrate. Our results suggest that the unusual high optimum temperatures with pNPP as a substrate is not due to the stability of the enzyme, since the behaviour was not maintained with other substrates which have been tested by Ferreira *et al.* (1998). In order to investigate the thermal stability of the enzyme in its two fractions the cell wall- bound and the secreted extracellular fractions and to elucidate any doubt that the substrate itself is sensitive to high temperatures, the pre-incubation treatment experiments on the enzyme have been

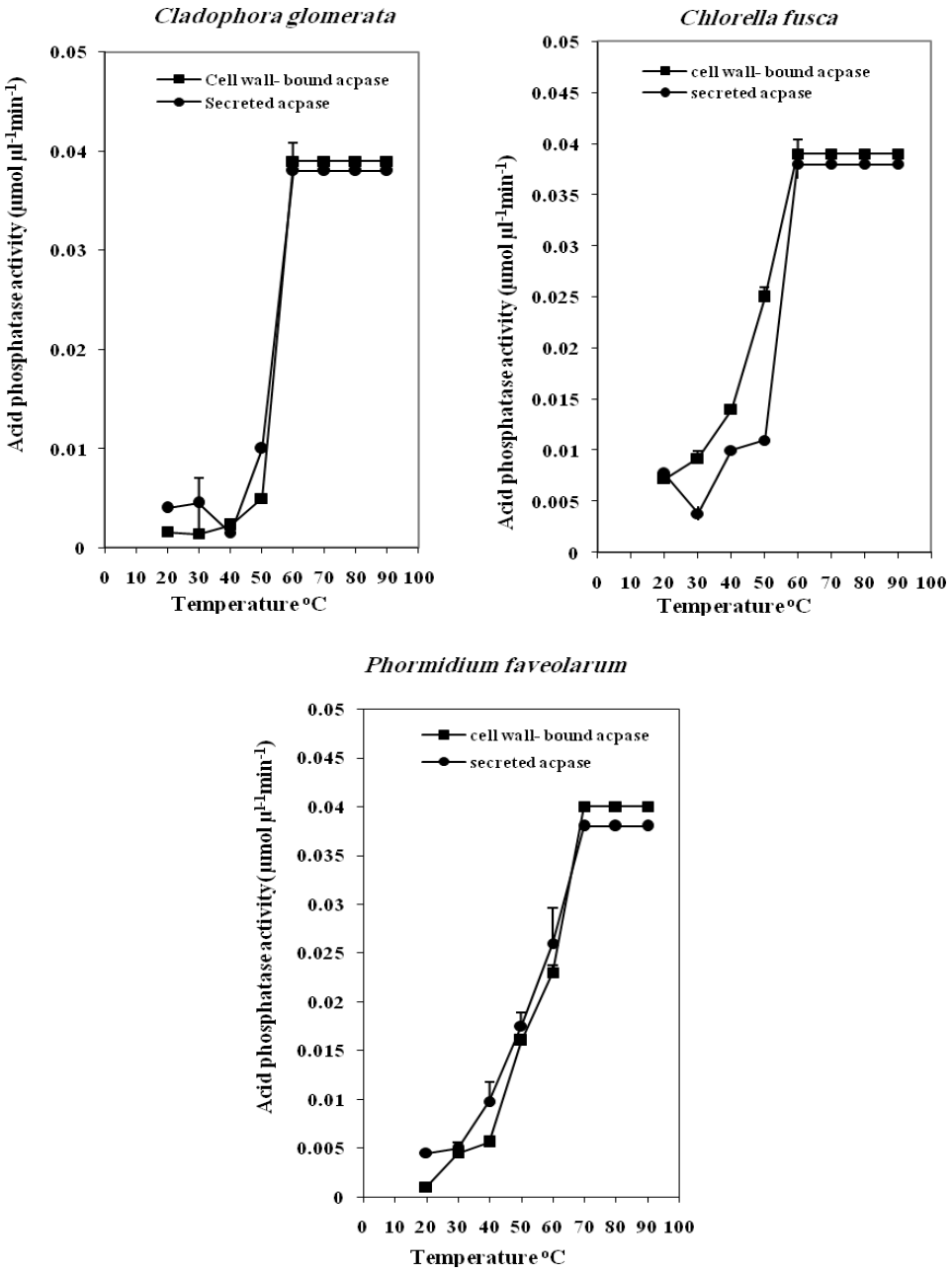

Figure 1. Effect of temperature on the activity of phosphatase enzyme of some algae for the wall bound and secreted fractions. Data are mean values ± SD, n= 3.

performed in absence of the substrate. It is clear from the results represented in figure (2) that the enzyme is stable even at high temperatures.

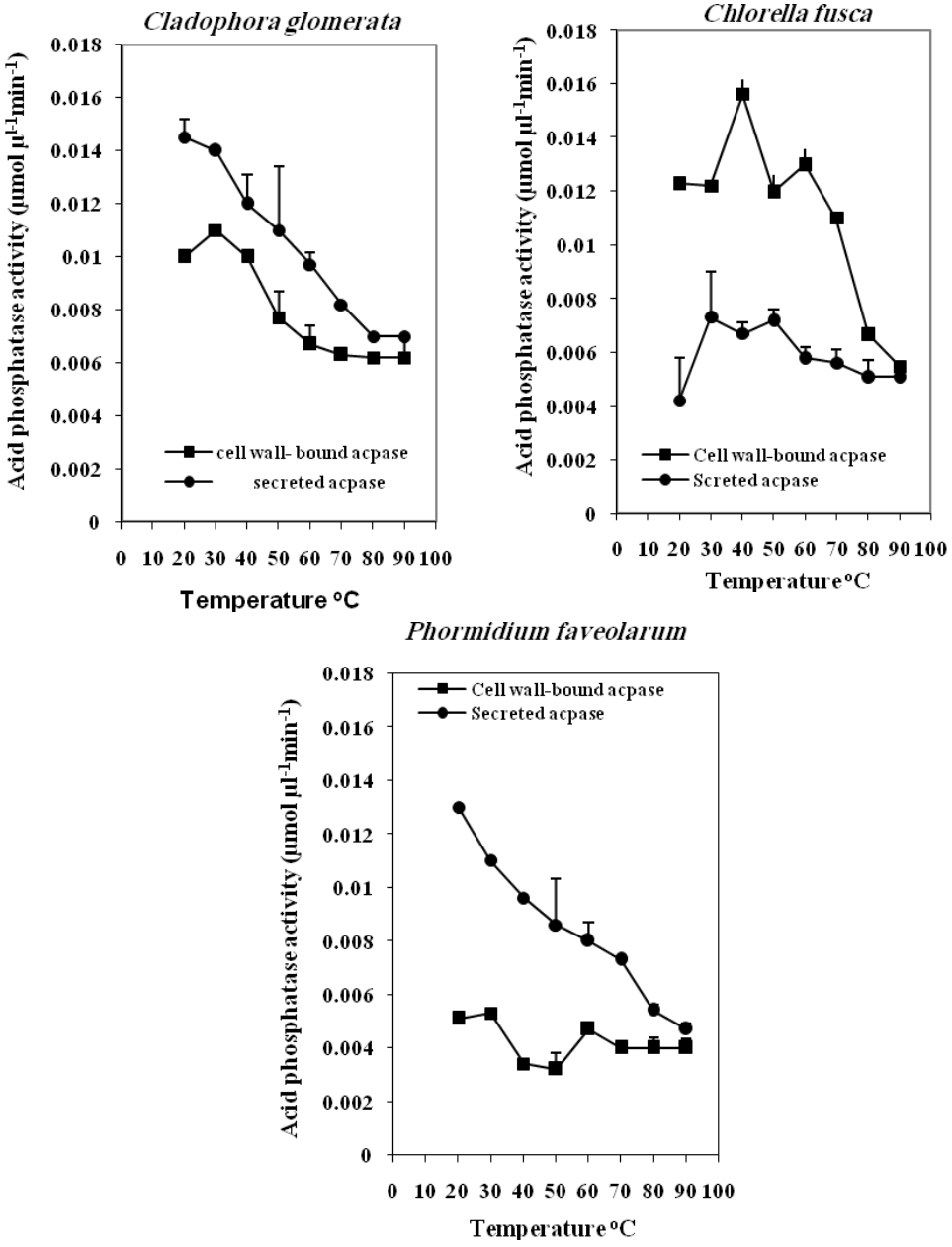

Figure 2. Thermal stability of the acid phosphatase enzyme of some alga for the cell wall- bound and secreted fractions. Data are mean values ± SD, (n= 3).

The protein (enzyme) completely undergoes all the thermal effects at the same optimum temperature obtained when using pNPP as a substrate in all the reactions of the experiment. The thermal behavior of the phosphatase enzyme isolated from the tested algae could tolerate the thermal inactivation with pNPP as a substrate and these results were parallel and in agreement with the results obtained by Park and Van-Etten (1986) and Ferreira *et al.* (1998).

In contrast to other phosphatases isolated from higher plants, the two fractions of the acid phosphatase isolated from the tested algae exhibited high stability at high temperatures even above 80 ºC whereas, at these temperatures other acid phosphatases showed significant loss of activity (Panara *et al*., 1990; Ibrahim *et al.*, 2002).

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دراسة لتأثير درجة الحرارة على نشاط انزيم الفوسفاتيز الحمضى المستخلص من *الكلوريللا فوسكا*، *الكلادوفورا جلوميراتا* و *الفورميديوم فافيولورم* و الثبات الحرارى للانزيم.

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تناول البحث دراسة تأثير درجة الحرارة على نشاط (كفاءة) انزيم الفوسفاتيزفى ثلاث أنواع من الطحالب تمثل ثلاث مجموعات مختلفة. و هم طحلب الكلوريللا فوسكا ممثلا للطحالب الخضراء وحيدة الخلية التى تعيش فى المياه العذبه، طحلب الكلادوفورا جلوميراتا و هو من الطحالب الخضراء الخيطية و الفورميديوم فافيولورم من مجموعة الطحالب الخضراء المزرقة الخيطية. و قد تبين من دراسة تأثير درجة الحرارة على النشاط الانزيمى أن درجة الحرارة المثلى لنشاط الانزيم بشقيه، المرتبط بالجدار الخلوى و المفرز خارج الخلية، كانت عند 60 درجة مئوية فى حالة طحلبى الكلوريللا فوسكا و الكلادوفورا جلوميراتا أما فى حالة طحلب الفورميديوم فافيولورم فقد كانت 70 درجة مئوية هى درجة الحرارة المثلى للنشاط الانزيمى.

و قد تناولت الدراسة أيضا السلوك الحرارى لانزيم الفوسفاتيزفى مستخلص من الطحالب المذكورة و الذى اتضح من خلاله أن الثبات الحرارى لا يقتصرعلى ثبات الانزيم فقط و انما يعزى الى استخدام البارا نيتروفينيل فوسفات كمادة للتفاعل أيضا. حيث انه قد تبين عمليا من خلال هذه الدراسة ثبات هذه المادة حراريا و عدم تحللها ذاتيا حتى بدرجات الحرارة المرتفعة. حيث أكدت الدراسة كفاءة هذه المادة للعمل كمادة للتفاعل مع انزيم الفوسفاتيز عند درجات حرارة أعلى من 80 درجة مئوية على عكس مواد التفاعل الأخرى التى اجريت عليها نفس الدراسة مع نفس الانزيم على الطحالب موضوع البحث.