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Influence of temperature on glutathione level and glutathione-related enzyme activities of Antarctic ice microalgae *Chlamydomonas* sp. ICE-L

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Abstract GSH system plays a role in the control of the redox balance state, anti-oxidation and protecting life from injury of ROS (reactive oxygen species). In present paper, the possible GSH system of *Chlamydomonas* sp. ICE-L has been investigated by evaluating GSH and GSH-related enzymatic responses at different temperatures using spectrophotometer methods. The results showed that the GSH system is correlated positively to low temperature, and other factors but GR are correlated negatively to high temperature. So GSH and GSH-related enzymes play an important role in the adaptation of Antarctic ice microalgae to low temperature.

Key words *Chlamydomonas* sp. ICE-L, Glutathione, Glutathione peroxidase, Glutathione reductase, Glutathione S-transferase, Acclimation to low temperature.

1 Introduction

Low temperature is a stress factor for most living organisms, and it can affect their growth. Changes of behavior, physiology, biochemistry and molecule will be induced by the low temperature. Plants will produce a great deal of reactive oxygen species (ROS) because of the increase of oxygen consumption under low temperature. Excessive ROS can inactivate the membrane fat, protein and nucleic acid, and even lead the cell dead. Organisms can control the ROS using the non-enzyme antioxidant component or enzyme antioxidant component, which are more important under stress. The glutathione system is a major member of them, and consists of reduced Glutathione (GSH), Glutathione peroxidase (GPx), Glutathione reductase (GR), Glutathione S-transferase (GST), and glutathione synthetase. GSH reduce H_2O_2 to H_2O, and then it change into GSSG with GPx. GSSG can be reduced to GSH by GR, which keep the redox balance state.

The changes of antioxidant enzymes activities were reported by many research groups in high plants under low temperature. GR and other antioxidant enzymes activities increased when general temperature plants such as *Pinus sylvestris* L., wheat, cucumber, rice, maize and spinach, adapted to cold. GSH level of spinach leaf also increased[1,2,3]. The anti-cold plants, such as maize, tomato, great red alga, *etc.*, have higher GR activity[2,4]. Antarctic organisms live in a very harsh natural environment characterized by low temperature, high dissolved oxygen concentration, strong seasonal changes in light intensity, strong radiation, and high salinity. These conditions could facilitate the produce of ROS. Regoli *et al.* (1997)[5] reported that the temperature had a little influence on the GR in gill of Antarctic scallop, but the scallop had a higher GST in its intestine. Antarctic ice algae is a great group of microalgae living in marine ice of the Antarctic pole. Recently the role of antioxidant enzymes in Antarctic ice algae on acclimating to ultraviolet radiation was testified. But the function of the GSH system on acclimation of Antarctic ice algae to low temperature has not been reported. In present study, the objective is to resolve the problem and to clarify adaptation mechanism of Antarctic ice algae more completely.

2 Materials and methods

2.1 Algal culture

A unialgal strain of Antarctic ice algae *Chlamydomonas* sp. ICE-L was obtained from the key lab of marine bioactive substance of State Oceanic Administration of China and cultured in the f/2 medium of Guillard and Ryther (1962)[6]. Triangle flasks containing 1200 mL medium were inoculated with 300 mL of a mother culture. The alga was grown at 6–8 ℃ in the refrigerator under a 12:12 light-dark cycle of 1300–1900 lux. Every flask was shaked 4–5 times a day.

2.2 Choice of temperature

The effects of temperature were investigated in f/2 medium under a 12:12 light-dark cycle of 1300–1900 lux. For this, *Chlamydomonas* sp. ICE-L of all groups were cultured for 2 days continuously with various temperature (including −10 ℃, 0 ℃, 8 ℃, 12 ℃ and 17 ℃) after they were cultured 7 days in same medium with the optimum temperature (8 ℃). Here the control group was at 8 ℃. Microalgae of all groups were sampled a time per 6 h from the beginning of day 8 and harvested for assessing the following parameters through centrifuging at 6000 rpm, and rinsed 3–4 times with distilled water.

2.3 Assays of GSH and GSH-related enzyme activities

Algal material were powdered in liquid nitrogen. These powdered materials were further homogenized in 5–10 times volume 50 mM Tris-HCl buffer (pH 7.0, including 20% (v/v) glycol, 1 mM ascorbate, 1 mM DTT, 1 mM EDTA, 5 mM $MgCl_2$ and 1% PVP). The extract was centrifuged at 6000 rpm for 10 min after freezing and thaw 3–4 times and the supernatants were used for analysis of GSH, GSH-related enzymes and protein.

Glutathione was measured on samples treated by 5% sulphosalicilic acid, centrifuged at 8700 rpm for 15 min. The resulting supernatants were assayed by the method of using DTNB (5,5′-dithio-bis(2-nitrobenzoic acid)). The GSH level was calculated by using the absorbance value at 412 nm according to the standard curve[7]. GPx was estimated as the decrease in absorbance at 412 nm according to change of GSH to GSSG when H_2O_2 was inverted to H_2O. One unit of enzyme activity represents 1 μM of GSH decreased min^{-1} mg^{-1} protein at 25 ℃. GST was assayed as the decrease in absorbance at 412 nm due to conjugation of GSH to CDNB (1-chloro-2, 4-dinitrobenzene). One unit of GST activity represents 1 μM of GSH decreased min^{-1} mg^{-1} protein under the assay conditions of 25 ℃. GR activity was determined by measuring the reduction of oxidized glutathione at 340 nm. The reduction of oxidized glutathione was measured by NADPH oxidation[8]. The reaction mixture contained 50 mM Tris-HCl (pH 7.5), 0.1 mM NADPH, 5 mM $MgCl_2$, 0.5 mM GSSG and 100 μL above extract in a final volume of 2.5 mL. One unit of enzyme activity represents 1 μM of NADPH oxidized min^{-1} mg^{-1} protein at 25 ℃. Protein concentrations were measured by the method of Bradford (1976)[9] using bovine serum albumin as a standard. All determinations are expressed as the mean ±SD of three separate experiments. SD value was calculated by Microsoft Excel 2000, and the significance test was valued by Duncan's new multiple range method of SPSS11.5 statistics software.

3 Results

3.1 GPx activity

GPx activity of the control group did not changed notably during the culture. On the other hand, the GPx activity of experimental groups changed significantly ($P < 0.01$) (Fig. 1). Compared to the control, GPx activity decreased at higher temperature, and increased at lower temperature. At 12 ℃ the lowest activity was 101.53 U, and the maximum was 299.53 U at 18 h. The minimum and maximum of GPx activity were 92.27 U and 212.80 U respectively at 17 ℃, and 529.90 U and 971.30 U respectively at 0 ℃, and 212.00 U (30 h) and 722.70 U (24h) respectively at − 10 ℃.

3.2 GST activity

The changes of GST were similar to GPx. The activity of the control was between 150.21 U and 170.80 U, and the experimental groups changed significantly (Fig. 2). Compared to the control, GST activity decreased at higher temperature. The nadir was 51.09 U, and zenith was 143.60 U at 12 ℃. But it increased at lower temperature than the control ($P < 0.01$). The nadir and zenith of GST activity was 302.62 U and 668.29 U (18 h) respectively at 0 ℃, and 299.48 U (30 h) and 751.64 U (18 h) respectively at − 10 ℃.

3.3 GR activity

GR activity of the control did not changed notably during the culture. But the activity of experimental groups, which maximum was all higher than that of the control, changed significantly ($P < 0.01$) (Fig. 3). At 12 ℃ maximum was 0.2791 U (30 h), and

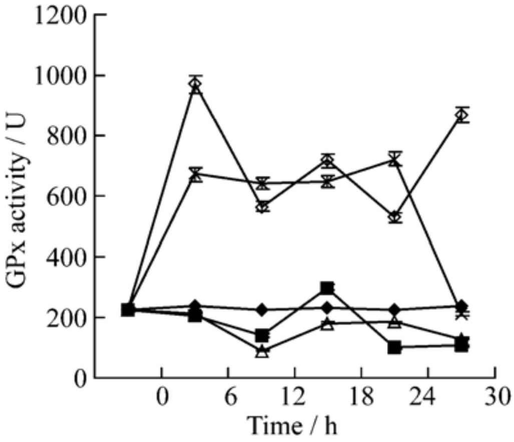

Fig. 1 GPx activity of *Chlamydomonas* sp. ICE-L under different temperatures. ◆ 8 ℃; ■, 12 ℃; △, 17 ℃; ◇, 0 ℃; ×, − 10 ℃.

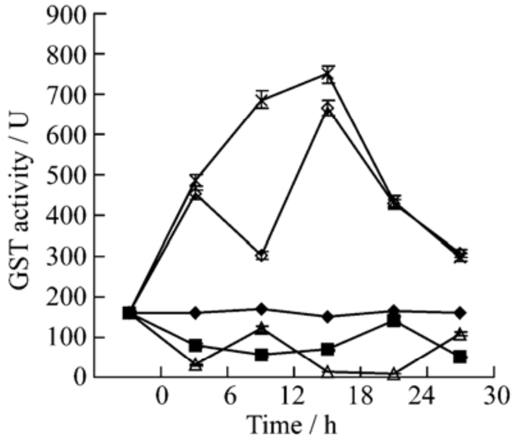

Fig. 2 GST activity of *Chlamydomonas* sp. ICE-L under different temperatures. ◆ 8 ℃; ■, 12 ℃; △, 17 ℃; ◇, 0 ℃; ×, − 10 ℃.

minimum was 0.1177 U (12 h) but still higher than that of the control. The activity reached the zenith (0.3417 U) in 6 h at 17 ℃, and then began to drop. At 0 ℃ maximum and minimum were 0.8336 U (6 h) and 0.0275 U (30 h) respectively. At last it was lower than the counterpart of the control. The maximum was 0.2395 U (12 h), and all higher than the control except at 30h at − 10 ℃.

3.4 GSH level

GSH level of the experimental groups changed significantly ($P < 0.01$). It can be seen decreasing above 8 ℃ (Fig. 4). The nadir of GSH level was 0.8176 nmol/μg and the zenith was found in 12 h at 12 ℃. But at 17 ℃ GSH level was higher than that of the control after 30 h. GSH content of the experimental groups below 8 ℃ was higher clearly than the control, and reached the zenith 2.5212 nmol/μg and 2.3980 nmol/μg after 6 h at 0 ℃ and after 18 h at − 10 ℃ respectively. At 0 ℃ the minimum appeared at 18 h, but it appeared with 1.5008 nmol/μg at − 10 ℃.

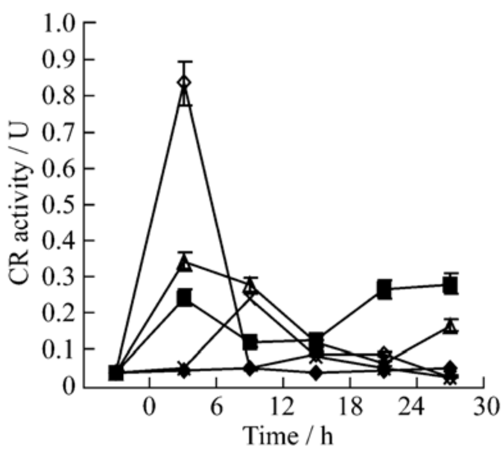

Fig. 3 GR activity of *Chlamydomonas* sp. ICE-L under different temperatures. ◆ 8 ℃; ■, 12 ℃; △, 17 ℃; ◇, 0 ℃; ×, − 10 ℃.

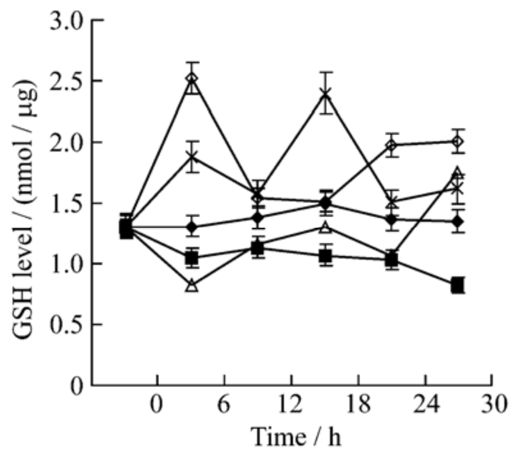

Fig. 4 GSH level of *Chlamydomonas* sp. ICE-L under different temperatures. ◆ 8 ℃; ■, 12 ℃; △, 17 ℃; ◇, 0 ℃; ×, − 10 ℃.

4 Discussion

It is well known that low temperature can induce producing of free radical and oxidation of membrane lipid in cells, and that activities of antioxidation enzymes are correlated to freezing tolerance of plant. GSH system confronts the active oxygen by many physiological ways. GSH related enzymes involved scavenging of ROS, preventing the peroxidation of lipid, and keeping the balance of GSH/GSSG.

GPx can clean out the lipid peroxide. GPx activity of rat tissue rose notably during cold exposure. Colin *et al* (2000)[10] discovered GPx increased by 43% when *Microtus agrestis* was moved from 22 ℃ to 8 ℃. This change was helpful to clean ROS at low temperature, and was relative to cold tolerance. Similarly, present results indicated that the GPx activity of the experimental groups was higher than that of the control when temperature was less than 8 ℃ (Fig. 1). It is obvious that increased GPx can scavenge overmuch ROS induced by low temperature, and protect ICE-L from harm. The relationship between GPx and cold adaption was hardly reported in plants and other algae. So it is clear that GPx is another factor involving in anti-cold stress of plant and agae. But the activity of GPx went down at more than 8 ℃. So ICE-L may clean ROS by other approachs in higher temperature stress.

GST activity in intestine of Antarctic scallop was much higher, and its GSH level and GST activity were higher under stress[5]. GST of cereal was positively correlated to cold tolerance, and it was higher significantly than that of the control in their leaf at low temperature. Like these data, in present results it was clearly found that GST of ICE-L rose when temperature went down, and it was related to the cold tolerance. Especially, the activity increased by 3 times at − 10 ℃ compared to that of control (Fig. 2). GST catalyses the conjugation of glutathione to a variety of electrophilic compounds and plays a role in the inactivation of toxic xenobiotics and their metabolites. GSTs are encoded by a poly-gene family, and they have many forms and a few different functions. Though all functions of GST were not assessed in this paper, it is incontestable that it is related to low temperature on the function of transferring thiol radical.

GR is important in alleviating the harm of ROS caused by cold stress. It was reported that GR in some plants increased during cold acclimation[2]. Expression of GR gene in wheat enhanced notably in 1week during adapting to cold[1]. GR was relative to the revival rate of soy seedling in 1week at 5 ℃. Anti-cold gene type of maize and tomato had a higher GR activity[2]. The antioxidant enzyme activities, enzymes protein level and gene expression of maize increased significantly at sub-optimum temperature. But the maize which was sensitive to chill had not plenty of GR[11]. Similarly, present results showed that GR activity of ICE-L was lowest at 8 ℃ (control), and increased quickly at low temperature (0 ℃ and − 10 ℃) (Fig. 3). It was obvious that increase of GR was relative to low temperature though their relationship was not completely positive. So GR of ICE-L has an important role in its cold adaptation. Following the quickening of consuming oxygen metabolism, ROS produced more quickly at low temperature. Enhancement of GR was helpful to clean free radical effectively and protect ICE-L cells. It was interesting that the GR would also increase above 8 ℃. This can be explained by the theory that slightly higher temperature was a kind of stress for ICE-L. Under the relative high temperature stress, a great deal of free radical also appeared in ICE-L cell. Increase of GR was resulted from the acclimation to high temperature, and it would protect the cells from injury. It was different with present results that GR activity of cucumber seedling root was relative to cold injury[12], and GR of cereal was not relative to freezing tolerance. This difference may be caused by different genus of organisms and experiment methods.

The cold-adapted GR isoforms had been discovered in pine, pea, maize, mustard and red spruce. They had a high activity under chill condition[14,15]. In this study, GR of ICE-L increased at low temperature possibly because of the expression of new GR isoform, or activation of intrinsic GR isoforms. Further study should be done on GR isoforms of ICE-L.

Higher level of GSH can protect the thiol radicals in protein, and decrease the formation of disulfur bond inside the molecule at low temperature. In present study, GSH concentration was higher significantly at 0 ℃ and − 10 ℃ than at 8 ℃. But GSH level dropped notably at 12 ℃ and 17 ℃ (Fig. 4). It was consistent with results of this paper that GSH level of freezing tolerance maize enhanced in a great extent under low temperature[2]. The same results were also found in tomato and wheat[15]. Increasing GSH involved in confronting free radical, thiol transferring, and stabilizing the enzymes. It also involved in tolerating low temperature indirectly through regulating the synthesizing of proteins and expression of genes[16].

In a word, it is suggested that the GSH system is correlated positively with the low temperature, and that other factors but GR are negatively to high temperature. GSH and GSH-related enzymes play an important role in the adaptation of Antarctic ice microalgae to low temperature.

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