

DETERMINATION OF OXIDATIVE STRESS PRECURSORS OF BLEACHED AND NON-BLEACHED ANEMONES FROM TURKISH COASTLINES

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Abstract

The antioxidant enzyme activities and the lipid peroxidation levels of bleached and non-bleached *Anemonia viridis* from Turkish coastlines were determined in the present study.

Keywords: Aegean Sea, Cnidaria

Introduction

Sea anemones, a group of the order Actinaria, show a symbiosis between cnidarian and an alga commonly known as zooxanthellae. This association is considered to be mutualistic as the two side of the association acquire some benefits from the presence of other side. Despite its benefits to each side, this mutualism must be carefully balanced. If this balance shifts towards to alga, the anemones will be destroyed by the excess O₂ and also the increased reactive oxygen species (ROS) from photosynthesis [1]. Due to their unpaired electrons, the reactive oxygen species react rapidly with some molecules in the body which may cause DNA injury, lipid peroxidation and protein carbonylation [2]. In order to protect themselves against the excess ROS, the anemones expel the alga from their bodies to reconstitute the balance between two sides of symbionts or the photosynthetic pigments of alga are removed. This alga or pigment loss cause whitening which is named "bleaching" in the anemone. There are some reports in the literature that elevated temperature, UV exposure, cold shock, pathogenic infections, reduced salinity, unfavorable light conditions or pollution may also be the other factors of bleaching [3]. To the best of our knowledge, this is the first report on the bleaching event of anemone species from the Turkish coastlines. The antioxidant enzyme activities and lipid peroxidation levels of the bleached and non-bleached anemones which were collected from the same locations were investigated in order to see the differences between these individuals.

Materials and Methods

The animals were collected from Dikili-Turkey in May 2009. 0.10 gram of samples was homogenized in 1 mL of phosphate buffer (pH 7.2, 50 mM) with Ultra-Turrax T8 IKA-Werke homogenizator. The homogenates were centrifuged in a refrigerated centrifuge (Hettich 32R) at 10,000 rpm for 10 min at +4°C to remove the cell debris and the supernatants were used in experiments. Total protein concentrations were determined by the Bradford Method using bovine serum albumin (BSA) as standard [4]. SOD activity was assayed by using a commercial kit produced by Randox (SD 125). CAT activity was determined by Aebi's method [5] that measures the decrease in absorbance of H₂O₂ at 240 nm. GSH-Px activity was assayed by using a commercial kit produced by Randox (RS 505). LPO level was measured by measuring the concentration of thiobarbituric acid reactive substances (MDA). The LPO level was calculated by using an extinction coefficient of 155 mM⁻¹cm⁻¹ as nmol MDA/g fresh weight of anemones [6]. Student t test was used to evaluate the experimental data. The statistical significance was considered as p<0.05.

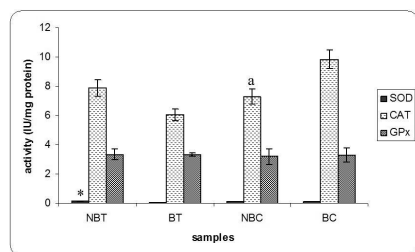


Fig. 1. Antioxidant enzyme activities of bleached and non-bleached anemones. NBT, BT, NBC and BC identify the non-bleached tentacles, bleached tentacles, non-bleached columns and bleached columns, respectively. * shows the statistical differences between the enzyme activities observed in the non-bleached and bleached tentacles. Letter (a) shows the statistical differences between the enzyme activities observed in the non-bleached and bleached columns. The results are the means of three different experiments and the error bars show \pm S.E.M

Results and Discussion

In order to evaluate if there is a connection between the bleaching of sea anemones and increased oxidative stress, the antioxidant enzyme activities were determined. The results were presented in Figure 1. SOD activities of non-bleached tentacles were statistically higher than the SOD activities of bleached ones (p<0.05). The excess H₂O₂ is scavenged by the activities of CAT. There was a statistically difference between the CAT activities of non-bleached and bleached columns (p<0.05). There was no statistical difference between the GPx activities of all species. No statistical differences were observed between the LPO levels of these species. According to the results of present study, there is no correlation between the antioxidant enzyme activities and the bleaching event.

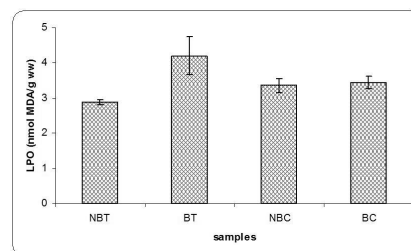


Fig. 2. LPO levels of bleached and non-bleached anemones. NBT, BT, NBC and BC identify the non-bleached tentacles, bleached tentacles, non-bleached columns and bleached columns, respectively. The results are the means of three different experiments and the error bars show \pm S.E.M

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