Ecotoxicology and Environmental Safety 151 (2018) 236–241

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Use of Cu/Zn-superoxide dismutase tool for biomonitoring marine environment pollution in the Persian Gulf and the Gulf of Oman

Farrokhzad Zeinali[a], Ahmad Homaei[b,*], Ehsan Kamrani[a,c], Seema Patel[d]

[a] Department of Marine Biology, Faculty of Sciences, Hormozgan University, Bandarabbas, Iran
[b] Department of Biochemistry, Faculty of Sciences, Hormozgan University, Bandarabbas, Iran
[c] Fisheries Department, Faculty of Marine Sciences, University of Hormozgan, Bandar Abbas, Iran
[d] Bioinformatics and Medical Informatics Research Center, San Diego State University, San Diego 92182, USA

ARTICLE INFO

Keywords:
Superoxide dismutase
Avicennia marina
Persian Gulf
Gulf of Oman
Biochemical properties
Environmental stressors

ABSTRACT

Superoxide dismutase (SOD) is the pivotal antioxidant enzyme that defends organisms against the oxidative stresses of superoxide radicals. In this experimental study, purification of SOD from the leaves of *Avicennia marina* (grey mangrove or white mangrove) from the family Acanthaceae, located in Sirik mangrove forest on the shore of the Gulf of Oman was performed, for the intended characterization of SOD. The Sirik *Am*SOD (*A. marina SOD*) expressed optimum activity in the pH range of 6–9 with the maximum activity at pH 8. The optimal temperature for Sirik *Am*SOD activity was 70 °C. Comparison of the pH and temperature optima in two regions (the Persian Gulf and the Gulf of Oman) showed significant differences with $P < 0.05$. The SOD from the Persian Gulf was more resistant against the environmental stressors, because of the biochemical adaption to this environment, which is harsher. The evidence from these results suggests that *Am*SOD has different characteristics in each place, and mangroves undergo different adaptations and require different protections. The results of the enzymatic research can be useful for ecological management of organisms.

1. Introduction

Mangroves are key ecological regions in the coastal forest of the tropics and subtropics habitats (Hibino et al., 2001). They have an important role in biogeochemical cycle as well as in economic activities (e.g. aquaculture and fishing) (Zahed et al., 2010). Mangroves have enormous ecological significance, as this is a habitat for numerous endangered organisms. Mangroves protect shorelines from erosion, provide ecotourism opportunities, and they possess high efficiency of carbon sequestration (Jing et al., 2015; Zeinali et al., 2017). Mangroves are widespread in 124 countries, located between 30°N and 30°S (Kuenzer et al., 2011). Unfortunately, rapid anthropogenic activities have put 35% of the world mangrove forests into depletion or destruction. Iranian mangrove forests are located in the north part of the Persian Gulf and Gulf of Oman, between the 25°11' and 27°52' parallels (Ghasemi et al., 2010). These mangroves are dominated by two species, *Avicennia marina* (grey mangrove, from the family Acanthaceae) and *Rhizophora mucronata* (red mangrove, from the family Rhizophoraceae), of which the former is dominant (Ghasemi et al., 2010; Zahed et al., 2010). *A. marina* is an evergreen shrub (Maguire et al., 2002; Fang et al., 2006).

The Persian Gulf has a climate which fluctuates from > 30 °C in summer to < 10 °C in winter. Annual rainfall is less than the evaporation rate, and there is no inflow of permanent fresh water. Consequently, Persian Gulf has high salinity, with a salt content of 38 − 50 g/L (Parvaresh et al., 2011). Petroleum hydrocarbons are detected in proximity (Zahed et al., 2010). The range of surface temperatures of water in the Sirik mangrove forest on the shore of the Gulf of Oman, varies from 23 °C in winter to 32 °C in the summer. The middle salinity of water is 38.6 g/L. The concentrations of heavy metals is low in the Sirik mangrove, because the fishing harbor is the only industrial activity present there (Keshavarz et al., 2013). Abiotic stressors such as temperature, salinity, osmotic and heavy metals impact the physiology of plants (Jabeen et al., 2011). The mangroves are unique among the plants because of their high morphological and physiological adaptations to extreme conditions (Kathiresan and Bingham, 2001). Their antioxidative pathway must be robust in order to survive in the extreme environment (Prashanth et al., 2008). Reactive oxygen species (ROS) are necessary evils in all the living organisms. ROS play offense and defense role in them. ROS is required in the regulation of cellular processes (Wang et al., 2016); however, excessive ROS can damage cell membranes and macromolecules such as

* Corresponding author.
E-mail addresses: a.homaei@hormozgan.ac.ir, a.homaei@gmail.com (A. Homaei).

https://doi.org/10.1016/j.ecoenv.2018.01.029
Received 19 November 2017; Received in revised form 9 January 2018; Accepted 11 January 2018

DNA, proteins and lipids (Li et al., 2015; Wang et al., 2016). So, organisms protect themselves from the injurious effects of ROS, by antioxidant systems (Wang et al., 2016).

Superoxide dismutase (SOD) is an ubiquitous antioxidant defense against the oxidative stress toxicity, which acts by converting toxic O_2^- (superoxide anion) into H_2O_2 and O_2 (Bruneaux et al., 2013)·H_2O_2 is further converted to H_2O and O_2 by catalases, peroxidases or peroxiredoxin (Grimm et al., 2015). SODs are metalloenzymes, the first defense weapon against oxidative stressors. SOD can occur in multiple isoforms, and are classified as per the type of metal at their active sites. Cu/Zn-SOD, Fe-SOD, Mn-SOD, Ni-SOD and Cu-SOD have recently been purified from the pathogen *Candida albicans* (Zeinali et al., 2015; Branco and Morais, 2016; Peterson et al., 2016). Cu/Zn-SOD (EC 1.15.1.1) is a homodimer, with the center of each monomer being occupied with a Cu and a Zn ion (Hemachandra et al., 2016). Cu/Zn-SOD are located in different places within the cell (Jabeen et al., 2011), and their diverse physiological roles have been proven (Anju et al., 2013; Švagelj et al., 2016).

We have previously studied the SOD from *A. marina* (*Am*SOD) in the Khamir mangrove forest (shore of the Persian Gulf) (Zeinali et al., 2017). In this study, the comparison of *Am*SOD from Sirik mangrove forest on the shore of the Gulf of Oman with *Am*SOD of the Persian Gulf has been carried out. The obtained results suggest that the *Am*SOD of the Persian Gulf mangrove plants possess better tolerance against the environmental stresses. This is because of the harsher environmental conditions prevalent in the Persian Gulf, and the plants growing their have to evolve strategies to counteract the stressors. Organisms adapted to extreme environments have proteins that show significant structural and functional differences (Capo et al., 1997). This study discusses the biochemical adaptations that enables mangrove plants to tolerate the environmental stressors. Also, this study demonstrates that the adaptations vary within a single species located at different places.

2. Materials and methods

2.1. Specimen collection and crude enzyme extraction

The samples of *A. marina* leaves from healthy plants were collected from the port of Sirik in the north shore of Gulf of Oman, Hormozgan province, Iran (Fig. 1). Subsequently, extracts were derived from the washed, crushed leaves. For details on the processing steps, the published paper on Khamir *Am*SOD can be referred to Zeinali et al. (2017).

2.2. SOD assays and protein measurement

SOD activity was assayed in 50 mM Tris-HCl buffer (pH 8.2, containing 1 mM EDTA (ethylenediaminetetraacetic acid) at room temperature), against 0.2 mM pyrogallol as substrate. To 50 µl of enzyme solution diluted in 2850 µl of Tris buffer, 100 µl of 0.2 mM pyrogallol was added and the inhibition of pyrogallol autoxidation to purpurogallin was measured for 5 min. The rate of autoxidation inhibition was assayed by monitoring the increment in absorbance at 420 nm. One unit of SOD activity is defined as the amount of enzyme required for 50% inhibition of the rate of pyrogallol autoxidation (Attar et al., 2006).

2.3. SOD characterizations

2.3.1. Optimum temperature

The optimum temperature for enzymatic reaction was optimized using 50 mM Tris-HCl buffer, and pH 8.2, at temperature ranges of 20–90 °C. The activity at optimal temperature was taken as 100%.

2.3.2. Optimal pH and pH stability

The pH optimum for the purified enzyme was assayed by analyzing its activity in the pH range of 2–12 using casein as a substrate and buffer systems of 50 mM sodium acetate for pH 2–6, phosphate for pH 7, Tris-HCl for pH 8–9 and sodium carbonate for pH 10–12. The relative activity (%) refers to the percentage of the initial reaction rate obtained by the enzyme in the maximum pH compere to other pH conditions.

2.3.3. Identification of the type of superoxide dismutase

In order to recognize Cu/Zn-SOD, Mn-SOD and Fe-SOD gels were exposed to different solution of KCN (0–20 mM) and H_2O_2 (0–20 mM). It is known that cyanide and H_2O_2 are inhibitors of Cu/Zn-SOD, while Mn-SOD is not responsive to these treatments, and Fe-SOD is only sensitive to H_2O_2 (Hammouda, 1999).

2.3.4. Statistical analyze

ANOVA (Analysis of variance) was used to find a significant difference in the SOD characteristics from samples collected from the two sampling sites (Davari et al., 2010). It was followed by a Duncan test, when ANOVA detected a significant difference. Non-homonymous script showed significant differences with $P < 0.05$. The results are reported as mean of 3 independent experiments ± SD. These analyses were performed using SPSS 19.0 (SPSS Inc.), a statistical software package.

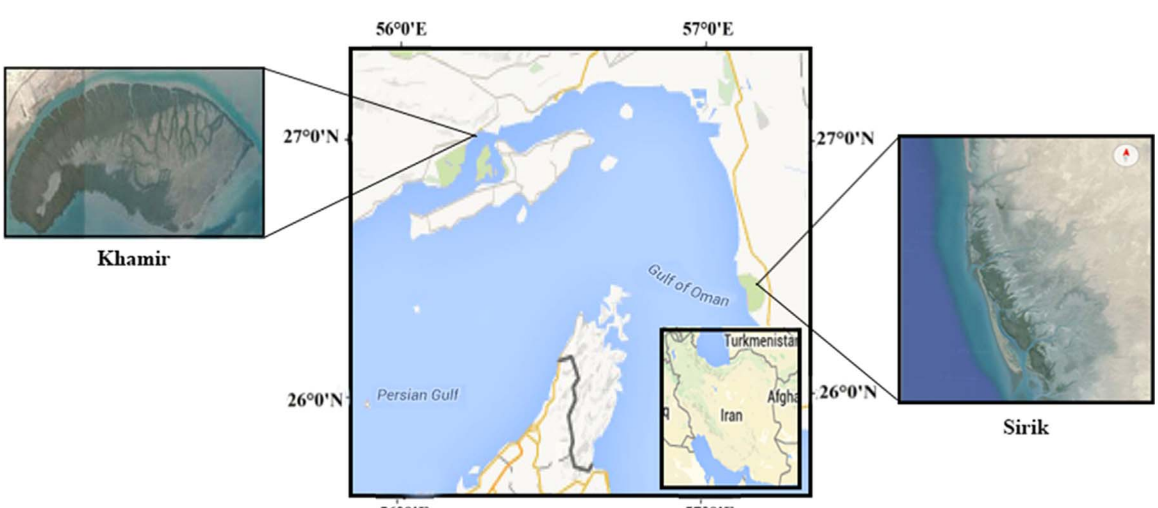

Fig. 1. The place of origin of the *Avecina marina* in Persian Gulf and Gulf of Oman. The samples come from port of Sirik in the north shore of Gulf of Oman, Hormozgan province, Iran.