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PARTIAL PURIFICATION OF CATALASE ENZYME FROM MUSCLE TISSUE OF DUSKY SPINEFOOT (*Siganus Luridus*)

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Abstract: Catalase, one of the antioxidant enzymes, decomposes hydrogen peroxide into water and oxygen. From the discovery of catalase, several studies have been carried out to reveal its importance in health, food and cosmetics industries etc., and these studies are still ongoing. In this study, catalase enzyme was partially purified from muscle tissue of Dusky spinefoot (*Siganus luridus*). Purification procedure consisted of homogenate preparation, ammonium sulfate precipitation and dialysis. The enzyme was precipitated in the range of 40-60 % Ammonium Sulfate concentration. The optimum buffer was determined as 200 mM phosphate buffer, optimum pH 8.0 and optimum substrate concentration 24mM for H_2 and O_2, respectively.

Keywords: Purification, Catalase, Characterization, Enzyme

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1. Introduction

Oxidative substances such as reactive oxygen species and free radicals and their undesirable biological effects are eliminated by enzymatic and non-enzymatic antioxidant defence systems. Enzymatic defence is provided by many enzyme systems such as glutathione peroxidase, glutathione reductase, superoxide dismutase, glutathione S-transferase, catalase and DNA repair enzymes. Non-enzymatic antioxidant defence systems include transferrin, lactoferrin, ceruloplasmin, uric acid, GSH and cysteine. Antioxidants are substances that can scavenge for free radicals and prevent cell damage (Shinda et al., 2012, Gelen et al., 2021, Ustundag et al., 2021).

The defence systems that function in the organism to prevent the formation of reactive oxygen species ward off the damage caused by these substances, and provide detoxification are called "antioxidant defence systems" or "antioxidants".

Catalase (CAT: EC 1.11.1.6) is a characteristic enzyme abundant in cells. This enzyme is widely present in animals, plants and microorganisms. It also plays an essential role in eliminating toxic hydrogen peroxide from cells (Masters et al., 1977).

Catalase enzyme catalyzes the removal of H_2O_2, one of the reactive oxygen species, which causes cellular damage, has a toxic effect and is one way to water. The enzyme uses H_2O_2 as a substrate, both as an electron acceptor and electron donor (Lanir and Schejter, 1975; Jones and Masters, 1976).

Siganus luridus is one of two species of siganus on Israel's Mediterranean coast. Both are migratory fish from the Red Sea described by Ben-Tuvia. This species migrated from the Red Sea along the Suez Canal to the Mediterranean, where it was first recorded in 1956 along the respective coasts of Israel. Later, they spread rapidly to the west and north and became widespread in Lebanon, the Turkish Republic of Northern Cyprus, the shores of Türkiye, Rhodes and the Central Aegean Sea. Since then, they have established significant populations in the Mediterranean and have acquired high economic importance. There are two species belonging to the *Siganidae* family in our country (*S. luridus* and *S. rivulatus*). The primary food of these two species in the *Siganidea* family is algae. The most defining feature that distinguishes these two types from each other is; *that S. luridus* has a caudal fin close to a flat shape, while *S. rivulatus* has a forked caudal fin. The length of both species can be around 25-30 cm. *S. luridus* has spines on its dorsal and ventral fins. These sharp and strong spines are covered with mucus mixed with venom and can cause painful wounds. They may lose their colour at dusk and change colour if threatened, but usually have a brown back, light brown abdomen and fine yellow stripes on both sides. They generally prefer coastal waters that are not deeper than 40 meters and on the rocks covered with vegetation. They live their entire lives without migrating to distant places or leaving their areas (Castriota and Andaloro, 2008).

2. Material and Methods

In this study, 8 grams of muscle tissue of the used Dusky spinefoot was weighed and used to prepare homogenate.

The muscle part taken from the fish was thoroughly pounded in a mortar with the help of liquid nitrogen and turned into flour, and the prepared homogenate was taken into a 50 ml tube and made up to 40 ml by adding 200 mM KH_2PO_4+0.5 mM EDTA+%PVP (pH: 8). It was then centrifuged for 20 minutes at 15000 g at 4 °C.

Solid ammonium sulfate precipitation process was applied to homogenates obtained from muscle tissue of Dusky spinefoot in the range of 0-20%, 20-40% and 40-60%, respectively. In this process, the homogenate is kept in ice and placed on a magnetic stirrer. Then, solid ammonium sulfate was carefully added to the homogenate at intervals of 45 seconds, not exceeding 1 gram. Centrifugation was performed for 10 minutes at 10000 g at 4 °C at each concentration interval.

Dialysis is performed to remove salts from the protein solution. In this study, dialysis was applied to remove the salt around the precipitated proteins at 40-60% ammonium sulfate concentration. For dialysis, 200 mM phosphate buffer was prepared, and the sample containing the precipitated proteins was placed into the membrane and left in the prepared buffer solution for 2 hours to remove the salts around the proteins.

Tris and phosphate buffers were prepared at 10mM, 50 mM, 100 mM, 200 mM and 300 mM concentrations, and activity measurements were made with these buffers at different concentrations.

In order to determine the optimum pH after buffer characterization, phosphate buffer was prepared at pH ranges of 5.5-6, 6.5-7, 7.5-8, 8.5-9 and activity measurement was made at these intervals.

In order to determine optimum substrate concentration, activity measurements were made for optimum substrate concentration using 3 mM, 6 mM, 12 mM, 18 mM, and 24 mM H_2O_2 substrate.

Determination of the optimum enzyme concentration was carried out by observing activity measurements using 25µl, 50µl, 100µl, 150µl and 200µl of enzymes.

3. Results

Ammonium sulfate was precipitated in the ranges of 0-20%, 20-40% and 40-60%, respectively, into the homogenate prepared for purifying catalase enzyme from the muscle tissue of Dusky spinefoot. In the results obtained after the activity measurements, it was determined that the value giving the highest activity was between 40-60% ammonium sulfate concentration.

KH_2PO_4 measurements were made at different concentrations to purify catalase enzyme from Dusky spinefoot muscle tissue. As a result of the activity measurements, it was determined that the most suitable ionic strength for catalase enzyme from stingray tissue was in 200 mM KH_2PO_4 buffer.

Catalase enzyme activity in shaded Dusky spinefoot muscle tissue was measured by spectrophotometer at pH 5.5-9.0 using 200 mM phosphate buffer. The optimum pH value of catalase enzyme obtained from Dusky spine foot muscle tissue was determined as pH 8.0 in 200 mM phosphate buffer.

The optimum substrate amount for catalase enzyme was measured using 200 mM KH_2PO_4 (pH 8.0) between 3-24 mM from Dusky spinefoot tissue. The optimum amount of substrate was determined as 24 mM.

4. Discussion

Catalase is one of the important antioxidant enzymes that significantly reduces oxidative stress by destroying cellular hydrogen peroxide to produce water and oxygen. It is assumed that catalase deficiency is associated with the pathogenesis of many age-related degenerative diseases such as diabetes, hypertension, anemia, vitiligo, Alzheimer's disease, Parkinson's disease, bipolar disorder, cancer and schizophrenia. Therefore, efforts are being made in many laboratories to investigate its use as a potential drug in treating such diseases (Nandi et al., 2019). In the process of partial purification of catalase enzyme from Dusky spinefoot (*Siganus luridus*) muscle tissue, the optimum ionic strength was determined in 200 mM potassium phosphate buffer. Maximum protein precipitation in ammonium sulfate precipitation was determined in the range of 40-60% concentration. Optimum pH: 8, optimum substrate amount was determined as 24 mM. When we compare the results of some studies in the literature on the purification process of the catalase enzyme; Optimum ionic strength was determined in 50 mM potassium phosphate buffer in the purification of catalase enzyme from hazelnut fungus (*Lactarius pyragalus*). Maximum protein precipitation in ammonium sulfate precipitation was determined at 60% concentration. Optimum pH determined as 8 (Sökmen and Ahıskalı, 2017). In the purification process of catalase enzyme from walnut (*Juglans regie*), maximum protein precipitation was determined at 40% concentration in ammonium sulfate precipitation process. Optimum pH is determined as 8 (Akar, 2015). In the purification process of catalase enzyme from celery (*Apium graveolens*), optimum ionic strength was determined in 50 mM potassium phosphate buffer. Maximum protein precipitation in ammonium sulfate precipitation was determined at 30% concentration. Optimum pH determined as 7.5 (Güngör, 2015). Our study is in accordance with the studies in the literature.

5. Conclusion

We aimed in this study for purification of catalase enzyme from Dusky spinefoot, determination of structural properties and characterization. We hope that results of this study will contribute to the elucidation of the function of the catalase enzyme in Dusky spinefoot, we think that our research can help future studies on catalase enzyme and antioxidant enzyme systems.

Author Contributions

The percentage of the author(s) contributions is present below. All authors reviewed and approved final version of the manuscript.

	B.Ç.	S.B.	D.E.
C	10	10	80
D			100
S			100
DCP	25	25	50
DAI	30	30	40
L	30	30	40
W	30	30	40
CR	30	30	40
SR	30	30	40
PM	30	30	40
FA	30	30	40

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans. In the study, muscle samples were used as experimental materials, which were used in the B.Sc. Graduation Thesis of first author entitled "Purification of the catalase enzyme from the muscle part of Dusky spine foot (*Siganus luridus*)" and stored at -80 °C. The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to.

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