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# Purification and Characterization of Black Porgy Muscle Cu/Zn Superoxide Dismutase

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**Chi-Tsai Lin, Tung-Liang Lee, Kow-Jen Duan and Jong-Ching Su (2001)** Purification and characterization of black porgy muscle Cu/Zn superoxide dismutase. *Zoological Studies* **40**(2): 84-90. Superoxide dismutase (SOD) has been proposed to be used as a bioindicator for environmental impact assessment. From a survey of SOD activity in black porgy, *Acanthopagrus schlegeli*, we found that Cu/Zn SOD was distributed rather evenly in 6 tissues, and in addition, only heart, liver, and testis had Mn SOD. We purified Cu/Zn SOD from muscle to homogeneity by a procedure that includes heating at 65 °C and fractionation on 2 chromatographic columns. The molecular mass of the native enzyme was 33 kDa and that of the subunit mass, deduced from a cDNA sequence, was 15.85 kDa. Thus the native enzyme appeared to be a homodimer. It had an N-terminal sequence of VLKAVCVLKGAGQTTGVV. The specific activity was 3318 u/mg. The enzyme had a broad optimum pH range of 5.8 to 11.2 and was resistant both to proteolysis by trypsin and chymotrypsin and to heat denaturation. The thermal inactivation rate constant of the enzyme at 80 °C was -0.0237 $min^{-1}$ and the half life for inactivation was 27.8 min.

**Key words:** Black porgy, *Acanthopagrus schlegeli*, Cu/Zn superoxide dismutase, Thermal stability.

Superoxide dismutase (SOD) catalyzes the dismutation of the superoxide ion ($O_2^-$) to hydrogen peroxide and oxygen molecule during oxidative energy processes. The reaction diminishes the destructive oxidative processes in cells. The level of scavenging enzymes has been extensively used as an early warning indicator of marine pollution (Buhler and Williams 1988). Recently, antioxidant enzymes have been proposed as bioindicators for environmental impact assessment (Livingstone 1991, Winston and Giulio 1991), due to the fact that both metals and certain organic xenobiotics generate oxidative stress (Sies 1986). Increased levels of several detoxifying and antioxidative enzymes have been described in molluscs and fish from the Spanish South Atlantic littoral in response to environmental pollution. This is particularly important in the Huelva Estuary, Spain, where the Tinto River brings Fe and Cu from pyrite mines, and organic xenobiotics such as industrial pollutants and pesticides are released. So, molluscs and fish caught in that zone show significant increases in SOD activity (Rodriguez-Ariza et al. 1991, Rodriguez-Ariza et al. 1992).

Based on such reasoning, the study of SODs and their application as biomarkers have become important areas in environmental impact assessment. SODs are metalloproteins and can be classified into 3 types, Cu/Zn, Mn, and Fe SODs, depending on the metal found in the active site (Brock and Walker 1980, Harris et al. 1980, Fridovich 1986). Cu/Zn SOD is predominantly associated with the cytosolic fraction of eukaryotes and is very sensitive to cyanide and hydrogen peroxide. Mn SOD is associated with mitochondria and is insensitive to cyanide and hydrogen peroxide. Fe SOD is found in prokaryotes and is not sensitive to cyanide but is inhibited by hydrogen peroxide. Previously, we cloned and se-

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