

ACTIVITIES OF SUPEROXIDE DISMUTASE AND CATALASE IN THE FOOT OF THREE FRESHWATER MUSSEL SPECIES. Branka R. Perendija¹, Slavica S. Borković¹, Tijana B. Kovačević¹, S. Z. Pavlović¹, Bojana D. Stojanović², M. M. Paunović², P. D. Cakić², Snežana B. Pajović³, and Zorica S. Saičić¹. ¹Department of Physiology, Siniša Stanković Institute for Biological Research, 11060 Belgrade, Serbia, ²Laboratory of Hydrobiology, Siniša Stanković Institute for Biological Research, 11060 Belgrade, Serbia, and ³Laboratory of Molecular Biology and Endocrinology, Vinča Institute of Nuclear Sciences, 11307 Vinča, Serbia

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All aerobic tissues continuously produce reactive oxygen species (ROS) during their respiratory activity. The resulting ROS flux can induce many cellular disturbances, such as depolymerization of polysaccharides and nucleic acids, oxidation of protein sulfhydryl groups, or peroxidation of fatty acids (Stojs et al., 2000). Antioxidant defense enzymes are important scavengers of these radicals. In aquatic organisms, two important antioxidant defense enzymes are superoxide dismutase (SOD) and catalase (CAT) (Perez-Campo et al., 1993).

The aim of our experiments was to determine the activities of total superoxide dismutase (Tot SOD, EC 1.15.1.1), copper and zinc-containing superoxide dismutase (CuZn SOD), manganese-containing superoxide dismutase (Mn SOD), and catalase (CAT, EC 1.11.1.6) in the foot of three freshwater mussel species: *Unio pictorum* (painter's mussel), *Unio tumidus* (swollen river mussel), and *Sinanodonta woodiana* (Chinese pond mussel). The first two species are native to Serbian freshwaters, but *S. woodiana* is an invasive species originating from Eastern Asia (Paunović et al., 2006).

The specimens (n=30, 10 of each freshwater mussel species) were collected from the Sava River, near the city of Šabac (44°46'17, 2°N and 19°42'16, 1°E), at an altitude of 70 m above sea-level in August, 2006. The foot of each mussel species was dissected, put in liquid nitrogen and then stored at -80°C until further biochemical analysis. Tissues were minced and homogenized in 5 volumes (Lionetto et al., 2003) of 25 mmol/L sucrose containing 10 mmol/L Tris-HCl (pH 7.5) at 4°C using an IKA-Werk Ultra-Turrax homogenizer (Janke and Kunkel, Staufen, Germany) (Rossi et al., 1983). The homogenates were sonicated for 30 seconds at 10 kHz on ice to release enzymes and then were centrifuged at 4°C at 100000 x g for 90 minutes (Takada et al., 1982). The resulting supernatants were used for biochemical analyses. Activity of Tot SOD was assayed by the epinephrine method (Misra and Fridovich, 1972) based on the capacity of SOD to inhibit autooxidation of adrenaline to adrenochrome. That of Mn SOD was obtained after inhibition of CuZn SOD with KCN. CuZn

SOD activity was calculated as the difference between Tot SOD and Mn SOD activities. CAT activity was measured from the rate of hydrogen peroxide decomposition (Claiborne, 1984). Total protein concentration was determined by the method of Lowry et al. (1951). All chemicals were purchased from Sigma (St. Louis, MO, USA). Antioxidant defense enzyme activities were expressed as specific (U/mg of protein) and total (U/g wet mass) as described by Barja De Quiroga et al. (1988). Data are given as the mean ± SE. Statistical significance between the species was analyzed using the unpaired t-test, a level of p<0.05 being considered significant (Hoeel, 1966).

A great number of biomarker studies have been performed on bivalves for the following reasons: their wide distribution, direct contact with the substrate, great tolerance to a huge variety of environmental conditions, and high bioconcentration of environmental toxicants due to intensive filtration activity. Table 1 presents data on the specific and total activities of Tot SOD, CuZn SOD, Mn SOD, and CAT in the foot of *U. pictorum*, *U. tumidus*, and *S. woodiana*. The obtained results show that specific activity of Tot SOD was significantly lower in *U. pictorum* than in *U. tumidus* (p<0.005) and *S. woodiana* (p<0.02). Also, the specific activity of CuZn SOD was significantly higher in *U. tumidus* than in *U. pictorum* (p<0.01) and *S. woodiana* (p<0.02). Our data show that Tot SOD and Cu Zn SOD activities expressed as total values were significantly higher in *U. tumidus* than in *U. pictorum* and *S. woodiana* (p<0.005). Total activity of Mn SOD was significantly higher in the foot of *U. tumidus* than in that of *S. woodiana* (p<0.005). Livingstone et al. (1995) reported that SOD in the mollusks seems to be a stable enzyme, rarely showing variations of activity. On the other hand, Orbea et al. (2002) investigated the influence of organic xenobiotics [polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenols (PCBs)] on SOD activity in *Mytilus galloprovincialis* and demonstrated the presence of seasonal variations in the activity of this enzyme. Regoli et al. (1997) detected higher SOD activity in gills of the Antarctic bivalve *Adamussium colbecki* compared to Medi-

Table 1. Activities of total superoxide dismutase (Tot SOD), copper and zinc containing superoxide dismutase (CuZn SOD), manganese- containing superoxide dismutase (Mn SOD), and catalase (CAT) expressed as specific (U/mg protein) and total (U/g wet mass) activity in the foot of *Unio pictorum* (Up), *Unio tumidus* (Ut) and *Sinanodonta woodiana* (Sw). Values are means \pm S.E. from 10 animals.

	<i>Unio pictorum</i>		<i>Unio tumidus</i>		<i>Sinanodonta woodiana</i>	
	Specific activity	Total activity	Specific activity	Total activity	Specific activity	Total activity
Tot SOD	11.57 \pm 0.64	464.05 \pm 11.15	14.79 \pm 0.49	591.76 \pm 16.79	13.58 \pm 0.42	451.79 \pm 11.00
CuZn SOD	7.79 \pm 0.44	311.65 \pm 13.13	10.65 \pm 0.43	425.80 \pm 14.19	9.43 \pm 0.40	313.96 \pm 12.30
Mn SOD	3.78 \pm 0.15	152.40 \pm 6.28	4.14 \pm 0.18	165.96 \pm 8.02	4.15 \pm 0.25	137.84 \pm 7.64
CAT	6.78 \pm 0.36	271.97 \pm 13.27	4.49 \pm 0.73	178.77 \pm 14.98	5.45 \pm 0.59	186.25 \pm 20.55

<i>Specific activity:</i>	Tot SOD: CuZn SOD: CAT:Up vs	Up vs Ut: p<0.005, Up vs Sw: p<0.02. Up vs Ut: p<0.01, Ut vs Sw: p<0.02. Ut: p< 0.005.
<i>Total activity:</i>	Tot SOD: CuZn SOD: Mn SOD: CAT:	Up vs Ut: p<0.005, Ut vs Sw: p<0.005. Up vs Ut: p<0.005, Ut vs Sw: p<0.005. Ut vs Sw: p<0.005. Up vs Ut: p<0.005, Up vs Sw: p<0.02.

terranean *M. galloprovincialis* and *Pecten jacobaeus*. Borković et al. (2005) found increased SOD activity in *M. galloprovincialis* collected in winter compared to specimens collected in spring. Some authors (Géret et al., 2002) found an increase of CuZn SOD activity in tissues of bivalves exposed to heavy metals. Also, induction of CuZn SOD isoform expression was described in the mussel *Mytilus edulis* exposed to contaminants in field and laboratory studies (Manduzio et al., 2003). SOD has been used as a biomarker for monitoring environmental pollutions (Nicholson and Lam, 2005; Jing et al., 2006). We found that specific and total CAT activity was significantly decreased in *U. tumidus* compared to *U. pictorum* (p<0.005), while total CAT activity was considerably lower in *S. woodiana* than in *U. pictorum* (p<0.02). CAT is regarded as an enzyme showing a clear and early response to contamination (Wenning et al., 1988). Previous reports suggested that CAT activity is elevated in mussels contaminated by PAHs (Porte et al., 1991). Livingstone et al. (2000) found increased CAT activity in the digestive gland of *M. edulis* after menadion treatment.

In conclusion, the present study represents the first comprehensive report on antioxidant defense enzyme activities in the foot of three freshwater mussel species from the Sava River. At the same time, the described experiments are the first to be performed on the non-indigenous species *S. woodiana*, from the Serbian part of the Sava River. The parameters employed in our work (SOD and CAT activities) can be useful tools in the biomonitoring of freshwater bivalves, as well as other freshwater organisms.

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