Electrochemistry of Superoxide Dismutases (SODs) and SOD-Based Third-Generation Biosensors for Superoxide Anion

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Superoxide dismutases (SODs) are ubiquitous metalloenzymes widely distributed in oxygen-tolerant organisms and generally involved in anti-cancer and anti-aging mechanisms. They protect the organism against the toxic effects of the superoxide by efficiently catalyzing its dismutation to O_2 and H_2O_2 via a cyclic oxidation-reduction electron-transfer mechanism. Considerable attention has been paid on electron transfer of SODs since information on the direct electron transfer is very useful in understanding the intrinsic thermodynamic and kinetic properties of the SODs and more importantly in practical development of the SOD-based third-generation biosensors for superoxide [1-4].

In the present work, we aimed at systematically studying the electrochemistry and electro-catalytic activity of the three kinds of commercially available SODs, i.e., Cu, Zn-SOD, Fe-SOD and Mn-SOD in SOD family, with gold electrodes modified with self-assembled monolayers (SAMs) of the so-called electron transfer promoters [5,6]. A large variety of thiols and disulfides were studied with respect to their capability to promote the direct electrochemistry of SODs. Experimental results strongly suggested that the electron-transfer (ET) properties of SODs remarkably depend on the structure of the promoters used, revealing the essence of structure-associated promoted ET properties of SODs. Among all the thiols and disulfides tested, the COOH-terminated aliphatic thiols and disulfides with a short alkylene chain [-$(CH_2)_n$-, (n = 1-3)] were found to be favorable for the ET of SODs (Fig.1).

The electrochemical properties of the SODs at the SAM-modified electrodes vary with the sort of SOD with respect to the formal potential, reversibility of electrode reactions, kinetic parameters (electron transfer rate constants and transfer coefficients) and pH dependence, suggesting different mechanisms for the electrode reactions of the individual SODs. A combination of the facilitated direct electron transfer and the bifunctional enzymatic catalytic activities of the SODs via a redox cycle of their active metals substantially offered a flexible electrochemical route to determination of $O_2^{\bullet-}$ where $O_2^{\bullet-}$ can be sensed with the SOD-based biosensors in both anodic and cathodic polarizations. Such an intrinsic feature of the SOD-based biosensors successfully enabled a sensitive determination scheme for $O_2^{\bullet-}$ free from the interference from some co-existing electoactive species, such as ascorbic acid (AA) and uric acid (UA).

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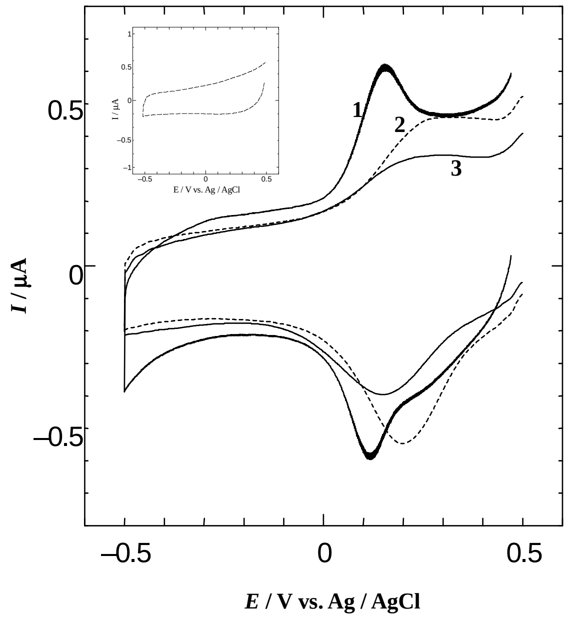

Figure 1. CVs at MPA (3-mercaptopropionic acid) –modified Au electrode in 5 mM PBS (pH 7.5) containing (1) Fe-SOD (0.32 mM), (2) Mn-SOD (0.40 mM) and (3) Cu, Zn-SOD (0.20 mM). Inset: CV of the MPA-modified Au electrode in PBS containing no SODs. Potential scan rate: 100 mV s^{-1}.