

Measurement of bicarbonate-dependent peroxidase activity of superoxide dismutase as monitored by ESR spin trapping and dichlorodihydrofluorescein oxidation

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The familial form of amyotrophic lateral sclerosis (FALS) has been reported to be associated, to some extent, with mutations in copper, zinc superoxide dismutase (SOD1). FALS-linked SOD1 mutants exhibit a gain-in-function that is linked to their toxicity. One of the aspects of the gain-in-function is increased peroxidase activity of SOD1 mutants. ESR spin-trapping technique was the first to show an increased peroxidase activity of FALS SOD-1 mutants. However, based on the increased signal intensity of DMPO-OH observed in the presence of SOD1 mutants, H₂O₂, and DMPO, the peroxidase activity was attributed to hydroxyl radical formation. In these studies, the role of bicarbonate anion was totally ignored. Our current view is that the increased peroxidase activity of SOD1 is related to the formation of carbonate anion radical, a potent yet selective oxidant. The carbonate anion radical generated at the active site of SOD1, H₂O₂, and bicarbonate anion could get out of the active site of SOD1 and oxidize several compounds including nitron spin traps and dichlorodihydrofluorescein (DCFH). Using the DCFH oxidation, we showed that spinal cord extracts of SOD1^{G93A} mice have increased peroxidase activity. The carbonate anion radical caused an increase in tyrosine nitration and aggregation of SOD1. Thus, we believe that bicarbonate-dependent peroxidase activity vis-a-vis the carbonate anion radical provides a unifying mechanism of toxicity of SOD1 mutants.