HYDROXYL RADICAL SCAVENGING ACTIVITY OF BIOLOGICAL SAMPLES WITH A MODIFIED CUPRAC METHOD USING A COLORIMETRIC SENSOR

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The hydroxyl radical (OH) emerges as a result of successive monovalent reduction of molecular oxygen as a part of cell metabolism, and being the most reactive species, it is primarily responsible for the free radical damage to tissues. Increasing attention has been directed to the discovery of antioxidants capable of scavenging hydroxyl radicals, and to the measurement of scavenging activities of such radicals. The idea here is to use an aromatic probe such as 2,4-dimethoxybenzoate for OH radical scavenging assay of a number of important biological compounds and biological fluids (e.g., tissue homogenate), make use of competition kinetics to simultaneously incubate the probe with the scavenger under the attack of hydroxyl radicals generated in a Fenton system, and measure the difference in CUPRAC absorbance of the probe in the absence and presence of the scavenger. A versatile colorimetric sensor was developed by impregnating Cu(II)-neocuproine reagent on a Nafion (C7H5F13O5S.C2F4) membrane for CUPRAC measurement. We used a 2,4-dimethoxybenzoate probe for detecting hydroxyl radicals generated from a stoichiometrically equivalent mixture of Fe(II)+EDTA with hydrogen peroxide. The produced hydroxyl radicals attacked both the probe and the antioxidants in 37 °C- incubated solutions for 1/2 h. The CUPRAC absorbance of the ethylacetate extract due to the probe decreased in the presence of OH scavengers, the difference being proportional to the scavenging ability of the tested compound. The second-order rate constants of the scavengers were determined by competition kinetics. The second order rate constants of biologically important S-compounds such as glutathione (GSH), cystine (CYS), and homocysteine (HCYS) using the developed method were 1.65x10^9, 2.75x10^9, and 1.66x10^9 M^-1s^-1, respectively. In addition to the tested compounds, kidney and liver tissue homogenate (in KCl solution) were evaluated for their OH scavenging activity using the developed method. Thus, a novel “test tube” hydroxyl radical scavenging assay has been developed using a modified CUPRAC method equipped with a colorimetric sensor.

References