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Reactivity of hydroethidine towards peroxyl radicals

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Because of the fact that the oxidation product of hydroethidine (HE), 2-hydroxyethidium (2-OH-E+), is an unique, fluorescent marker for superoxide radical anion (O2•-), hydroethidine has become one of the most used fluorogenic probes for its intracellular detection. Although, HE can be used rather as a qualitative than quantitative probe for detection of superoxide, it is still worthwhile to examine the mechanism of its oxidation, and factors affecting formation of 2-OH-E+ [1].

Here we present results showing the reactivity of hydroethidine towards chloromethylperoxyl radicals like CH2ClO2•, CHCl2O2• and CCl3O2•, generated in oxygen saturated, water/propan-2-ol solution containing 4% (v/v) of proper halocarbon compound. We showed that chloromethylperoxyl radicals are able to oxidase HE with the second-order rate constant at pH=7.4 of k=2.74 x 108 M-1s-1, k=8.8 x 108 M-1s-1 and k=1.23 x 109 M-1s-1, for corresponding radicals, measured by pulse radiolysis. Spectral characteristic of the product formed upon one-electron oxidation of HE by RO2• radicals obtained under mentioned conditions was the same with that obtained from the irradiation of HE embedded in 1-butyl-3-methylimidazolium hexafluorophosphate (BMIM+PF6-) low-temperature glasses and characterized as hydroethidine radical cation (HE•+).

The result were concluded by theoretical calculation using TD-DFT um052x/6-311+g(d,p) basis set. Results were depicted as an electrostatic potential and spin densities maps of hydroethidine radical cation (HE•+) and other species, which formation is highly possible under mentioned experimental conditions.

[1]. Zielonka J., Vasquez-Vivar J., Kalyanaraman B. Nat. Protoc. (2008) 3, 8

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