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Meeting abstract

H_2O_2 detection with 10-acetyl-3,7-dihydroxyphenoxazine: comparison with homovanillic acid

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H_2O_2 is assumed to be produced and involved in several (patho-)physiological processes. For the determination of low amounts of H_2O_2 formed in biological systems sensitive and reliable assays are necessary. Exploring suitable detection systems for mitochondrial H_2O_2 production different enzyme-catalyzed redox reactions were tested. By horseradish peroxidase (HRP) and H_2O_2, homovanillic acid (HVA) and 10-acetyl-3,7-dihydroxyphenoxazine (Amplex Red) were enzymatically oxidized to the fluorescent HVA dimer (λ_{ex} = 312 nm; λ_{em} = 420 nm) and resorufin (λ_{ex} = 563 nm; λ_{em} = 587 nm), respectively. The specificity of the assays was confirmed by catalase which dose-dependently inhibited the H_2O_2-induced fluorescence increase. Amplex Red and HVA were applied to the H_2O_2-generating glucose/glucose oxidase system and compared for their sensitivity. Albumin, which is frequently added to mitochondrial or cell incubation media, significantly decreased the fluorescence intensity of the Amplex Red oxidation product while the fluorescence intensity of the HVA dimer was significantly increased. This effect was observed in 0.3 M sucrose and was lacking in 0.15 M KCl. The mitochondrial H_2O_2 formation was studied in antimycin A-inhibited succinate-respiring rat heart mitochondria in the presence and absence of superoxide dismutase which catalyzes the dismutation of primarily produced superoxide radicals to H_2O_2. Amplex Red turned out to be a more sensitive analytic tool for H_2O_2 detection than HVA.