ESR spin-trapping of a carbon-centered free radical from agonist-stimulated human platelets.

許準榕
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Abstract

Several free radical intermediates formed during synthesis of prostaglandin H synthase (PGHS) catalyze the biosynthesis of prostaglandins from arachidonic acid (AA). We attempted to directly detect free radical intermediates of PGHS in cells. Studies were carried out using human platelets, which possess significant PGHS activity. Electron spin resonance (ESR) spectra showed a g = 2.005 signal radical, which was formed by the incubation of collagen, thrombin, AA, and a variety of peroxides with human platelets. The ESR spectra obtained using 5,5-dimethyl-1 pyrroline N-oxide (DMPO) and alpha-phenyl N-tert.-butylnitron (PBN) were typical of an immobilized nitroxide. Extensive Pronase digestion of both the DMPO and PBN adducts allowed us to deduce that it was a carbon-centered radical. The formation of this radical was inhibited by potassium cyanide and by desferroxamine. Peroxides stimulated formation of the g = 2.005 signal radical and inhibited platelet aggregation induced by AA. PGHS cosubstrates increased the intensity of the radical signal but inhibited platelet aggregation induced by AA. Both S-nitro-L-glutathione and reduced glutathione quenched the g = 2.005 radical but could not restore platelet aggregatory activity. These results suggest that the carbon-centered radical is a self-destructing free radical formed during peroxide-mediated deactivation of PGHS in human platelets.