Peroxidase Activity of Cytoglobin: Biochemical Characterization

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Introduction. Cytoglobin (Cygb) is a member of the globin superfamily. Cygbis abis-histidinehexacoordinatedheme protein present in humans and other vertebrates. The biological role played by Cygb is not well established and different functions have been postulated. The presence of a bis-histidine hexacoordinatedheme iron as the redox center was thought to impair the peroxidase activity of Cygb, differently of the pentacoordinated myoglobin and the hexacoordinated cytochrome c with a labile sixth ligand (Met80). **Objective.** In the present study it was investigated the reactivity of Cygb with hydrogen peroxide (HOOH), *tert*-butylhydroperoxide (*t*-BuOOH) and cumenehydroperoxide (*Cu*OOH). **Methods.** The peroxide-mediated conversion of Cygb to high valence species was analyzed by UV-visible and by spin trapping of free radicals and direct continuous wave EPRof heme iron. **Results.**Cygb was assayed with different concentrations of peroxides and spectra were run at different times. The intensity of the Soret band at 415 nm was determined at different times and the initial rate of the reaction plotted versus peroxide concentrations. The constants for *Cu*OOH, *t*-BuOOH and HOOH were $6.367.10^{7}\pm 6.10^{6}M^{-1}s^{-1}$,$2.70.10^{7}\pm 6.10^{6}M^{-1}s^{-1}$,$1.2.10^{7}\pm 1.10^{7}M^{-1}s^{-1}$, respectively. The reactivity of Cygb with peroxides was corroborated by EPR measurements, Cygb was converted to the high valence species by peroxides, promoted homolytic scission of the O-O bond and generated different radical from the different peroxides. EPR spectra obtained in the course of the reactions corroborated the conversion of the Cygb hexacoordenated heme iron to compound II. **Conclusion**: Cygb reacts with peroxides and produces free radicals and the catalytic efficiency is modulated by the access of the peroxide to the heme iron. These results are consistent with a biological role of Cygb grounded on pro-oxidant signaling activity.

Keywords: cytoglobin, EPR, recombinant protein, peroxidase activity

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