2014

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Free Radical Biology & Medicine |d New York, v. 76, Suppl. 1, p. S138, Dez. 2014
http://www.producao.usp.br/handle/BDPI/48900

Chemistry and Reaction Mechanisms

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Reduction of Hexacoordinate Globins by Cytochrome B5

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Since 2000, several hexacoordinate globins have been discovered. The function of these proteins is still a matter of debate. In general, hexacoordinate globins autoxidize at rates much faster than these of typical oxygen carrier proteins such as hemoglobin and myoglobin. As most putative functions of these proteins involve the ferrous form (Fe^{2+}) or the ferrous oxy form (Fe^{2+} O_2), the biological activity of these proteins may require the presence of a suitable reducing system.

In the case of hemoglobin/myoglobin, the cytochrome b5/cytochrome b5 reductase system converts ferric hemoglobin (methemoglobin) back to ferrous hemoglobin. Based on the structural similarity of globin X, neuroglobin, and cytoglobins to hemoglobin, we hypothesize that cytochrome b5 can effectively reduce these proteins from the ferric form to the ferrous form.

To test this hypothesis, we reacted oxidized globins with cytochrome b5, b5 reductase, and NADH under anaerobic conditions. Specifically, we examined human cytoglobin, and the zebrafish proteins cytoglobin 1 and 2, neuroglobin, and globin X. Globins were oxidized with excess potassium ferricyanide, which was subsequently removed with a filtration column. Oxidized globins were then mixed with b5 and b5 reductase. Following addition of NADH, spectra from 450-700 nm were measured for several minutes to monitor the extent of reduction.

The data gathered indicate that cytochrome b5 is capable of reducing all globins tested. The hexacoordinate globins were reduced at rates comparable to, if not faster than, methemoglobin. This work provides evidence to suggest that the physiologic b5 reductase system may be able to preserve these globins in the reduced state *in vivo*.

doi: 10.1016/j.freeradbiomed.2014.10.105

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Kinetic Characterization of Sulfenic Acid Reduction in 1-Cys Peroxiredoxins by Ascorbate

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Peroxiredoxins (Prxs) are Cys-based peroxidases with remarkable catalytic efficiency that can be divided into 1-Cys or 2-Cys, depending on the number of Cys residues involved in catalysis. Initially, reduction of Prx was described to be strictly dependent on thiols, but later we showed that ascorbate can also reduce the sulfenic intermediate of 1-Cys Prx (1-Cys Prx-SOH) in various organisms [1]. Here, the kinetic characterization of 1-Cys Prx-SOH reduction by ascorbate is described. Reduction of 1-Cys Prx-SOH by ascorbate was initially analyzed using an enzyme from *A. fumigatus* (AfPrxA) that is 37% similar to PRDX6 (human 1-Cys Prx). H_2O_2 levels were determined by means of a specific electrode (Free Radical Analyzer 4100), using a steady-state bi-substrate approach. AfPrxA decomposed H_2O_2 with good efficiency (k_{cat}/K_M = 7.4 x$10^3M^1.s^1$), through a Bi-Bi Ping-Pong mechanism. To further support these findings, a second, independent approach was also employed: competition between dichlorophenolindophenol (DCPIP) and AfPrxA-SOH for ascorbate. DCPIP is a redox sensor, whose blue color is lost when reduced and its second-order rate constant with ascorbate is 718 $M^1.s^1$, enabling the determination of the rate constant of the reaction between AfPrxA-SOH and ascorbate: 1.5 x $10^3M^1.s^1$. Therefore, by two independent approaches, we showed that ascorbate efficiently reduced AfPrxA-SOH. Next, the reductions of 1-Cys Prx SOH in other organisms (bacteria, yeast and plant) were also investigated and again the constants were in the 10^3M^1.s1 range. We conclude that the reduction of 1-Cys Prx-SOH by ascorbate is probably relevant in the subcellular compartments in which this reductant is present at millimolar levels. We are currently studying the reduction of 1-Cys Prx-SOH by ascorbate in other proteins, which could open new perspectives in cellular redox processes in vivo.

[1] Proc.Natl.Acad.Sci. USA. 2007 104:4886-91

doi: 10.1016/j.freeradbiomed.2014.10.106

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Site-Specific Radical Formation in DNA Induced by the Potent Oxidizing Agent HOCL, Using ESR, Immuno-Spin Trapping, LC-MS and MS/MS

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Hypochlorous acid (HOCl) is a powerful oxidant generated by the myeloid cells and is likely to contribute to damage mediated by these inflammatory cells. It is a major end product of oxygen metabolism in activated phagocytes and thus its reaction with cellular constituents is of biochemical and clinical interest. The haem enzyme myeloperoxidase, catalyzes the production of HOCl, from hydrogen peroxide and chloride. In addition to its physiological source, HOCl can also be generated by chlorine gas from accidents or a potential terrorist attack. HOCl can cause extensive damage to macromolecules like DNA. In this study we examine the ability of HOCl to damage DNA using spin-trapping, ESR and MS and MS/MS. The radicals generated are trapped by DMPO immediately upon formation. The DMPO adducts formed are initially EPR active but are subsequently oxidized to the stable nitrone, which can then be detected and visualized by IST and further characterized by MS and MS/MS. Here we report the DNA radicals (from cytosine, guanosine and adenine bases) detected from the treatment of DNA with either endogenous or exogenous HOCl in the presence of DMPO.

doi: 10.1016/j.freeradbiomed.2014.10.107

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Mechanism of the Reaction of Peroxynitrite with Mn-Superoxide Dismutase: Nitration of Critical Tyrosine-34

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