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The Protein Tyrosine Phosphatase (PTP) family of phosphatases is involved in normal cell functions, as well as in disease states such as diabetes and cardiovascular diseases. We previously reported on the role of the phosphotyrosine recognition loop of PTP1B in protein-protein interactions (Londhe *et al.* 2020, Nat. Chem. Biol., 16(2):122-125) and showed that interfering with protein-protein interactions between PTP1B and 14-3-3z using a pan-14-3-3 inhibitor destabilizes the reversibly oxidized form of PTP1B (PTP1B-OX) and activates the phosphatase. We rationally designed peptides to determine whether selective and effective activation of PTPs was possible. We first performed a structural analysis to compare the changes occurring in PTP1B-OX compared to its reduced form. Using this approach, we observed profound conformational changes in the phosphotyrosine recognition loop of PTP1B and rationally designed peptides comprised of the sequence of amino acids of the newly exposed loop, with a TAT (transactivator of transcription) peptide sequence in amino or carboxy-terminal to facilitate cell permeability. The treatment of cells with these peptides prevented the interaction between PTP1B-OX and 14-3-3z and maintained PTP1B in its active form. Interestingly, destabilizing the PTP1B-OX form by treating cells with rationally designed PTP1B activator peptides also decreased EGFR phosphorylation at PTP1B sites, without affecting other 14-3-3 signaling such as NOX-dependent production of superoxide and intracellular hydrogen peroxide. Moreover, exposing epidermoid carcinoma cells to PTP1B activator peptides prevented EGFR phosphorylation and impaired colony formation. Collectively our data suggests that PTP1B activator peptides may have potential as drug leads.

doi: https://doi.org/10.1016/j.freeradbiomed.2020.10.081

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Permeability of Lipid Membranes to Hydrogen Peroxide

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Hydrogen peroxide (H_2O_2) is an oxidizing species produced by several enzymatic mechanisms. It has many physiological functions including defense against pathogens, regulation of cell growth, migration and proliferation. It has been shown that cell membrane permeability to H_2O_2 depends on the presence of specific aquaporins, but there is no data on simple diffusion through pure lipid membranes. In this work we set out to determine the permeability coefficient (Pm) of liposome membranes to H_2O_2 through an enzyme latency method, using liposomes with trapped catalase. Preliminary results indicate that the permeability of membranes composed of DMPC, DOPC, DPPG, and cholesterol in different proportions varies between 2.0×10^{-5} cm/s and 1.3×10^{-3} cm/s.

The partition coefficient between organic solvents (octanol, hexadecane and olive oil) and water was also determined, as well as the thermodynamics of the process. This gives us an idea of the solubility profile of H_2O_2 at different membrane depths. The partition coefficient was found to be 0.07 in octanol vs water, and it decreased 4 orders of magnitude in hexadecane vs water. The distribution in organic solvents is thermodynamically unfavorable, mainly because of the entropic component, consistent with a hydrophobic effect on H_2O_2. Therefore, the main barrier to the transport of H_2O_2 through the membrane is thermodynamic and is because of the low solubility of H_2O_2 in the hydrophobic fraction of the bilayer. These results will be useful for understanding the permeability of different cellular membranes to H_2O_2 and distinguish the relative importance of the lipid fraction and the protein channels in H_2O_2 diffusion.

doi: https://doi.org/10.1016/j.freeradbiomed.2020.10.082

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Oxygen-independent Generation of Hydrogen Peroxide in Water Irradiated by Carbon-ion Beam

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The oxygen independent formation of hydrogen peroxide (H_2O_2) in an aqueous solution during carbon-ion beam irradiation was demonstrated. The radiation-induced hydroxyl radical ($^{\bullet}OH$) generation in an aqueous solution was reported to occur in two different localization densities, which were at the milli-molar (relatively sparse) and/or molar (super-dense) levels. In the milli-molar-level $^{\bullet}OH$ generation atmosphere, $^{\bullet}OH$ generated at a molecular distance of 4.3–6.6 nm are unlikely to interact with each other. However, in the molar-level $^{\bullet}OH$ generation atmosphere, several $^{\bullet}OH$ were generated with a molecular distance of 1 nm or less, and two $^{\bullet}OH$ can react to directly make H_2O_2. An aliquot of ultra-pure water was irradiated by 290 MeV/nucleon carbon-ion beam at the Heavy-Ion Medical Accelerator in Chiba (HIMAC, NIRS/QST, Chiba, Japan). Irradiation experiments were performed under air or hypoxic (<0.5% oxygen) conditions, and several linear energy transfer (LET) conditions (20, 40, 60, 80, or >100 keV/μm).
H_2O_2 generations in irradiated samples were estimated by two methods below. 1) The $^{\bullet}OH$ synthesized from H_2O_2 by UVB irradiation was spin-trapped with DMPO, and the $^{\bullet}OH$ adduct of DMPO (DMPO-OH) was then measured as an index of H_2O_2 using an X-band EPR. 2) A red quinoid dye (absorbance at 505 nm) formed by a reaction of 4-aminoantipyrine and phenol and H_2O_2 under coexisting peroxidase were measured using the spectrophotometer. Amounts of H_2O_2 generation per dose was estimated.