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Presence of Ser instead of Thr in the Catalytic Triad of Typical 2-Cys Prx Increases their Resistance to Hyperoxidation and Inactivation

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Typical 2-Cys Prx (Prx1/AhpC sub-family) are highly efficient peroxidases that uses a reactive cysteine (peroxidatic cysteine = C_P) to decompose hydroperoxides and display an intermolecular disulfide between C_P and the resolving cysteine (C_R) as a catalytic intermediate. Remarkably, 2-Cys Prx switch between distinct oligomeric states in a redox dependent manner. When the C_P is reduced or hyperoxidized, a decamer (pentamer of homodimers) is stabilized, which dissociate into dimers upon disulfide formation. As consequence of heat shock or C_P hyperoxidation, these enzymes may acquire molecular chaperone function (holdase). The high reactivity of 2-Cys Prxs is related to polar interaction among residues that compose the catalytic triad (Thr/Ser, Arg and C_P). Despite the fact that C_P and Arg are strictly conserved, the catalytic Thr can be naturally replaced by Ser in some 2-Cys Prx enzymes. We have shown before that the presence of a Ser stabilizes the decameric form of 2-Cys Prxs independently of their redox state. Using the typical 2-Cys Prx (Tsa1 and Tsa2) from yeast and proteins carrying Thr/Ser reciprocal substitutions, we demonstrated that enzymes containing Ser are more resistant to C_P hyperoxidation. Analysis of all typical 2-Cys Prx sequences available revealed that Ser is noticeably more common in bacteria than in eukaryotes. Through kinetic and structural analysis, we confirmed that substitution of Thr by Ser confers resistance to hyperoxidation also in bacterial enzymes (AhpC), which may be related with the enhanced stability of the decamers, as determined by thermal shift approaches. Finally, we observed that an AhpC containing Ser in active site is more efficient as molecular chaperone (holdase) by preventing thermal aggregation of citrate synthase. Therefore, the natural substitution of Thr/Ser within the catalytic triad produce striking differences in the biochemical and structural features of typical 2-Cys Prx whose biological significance is under investigation.Supported by FAPESP Redoxome (2013/07937-8)

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Chloroacil Hydroquinone Modulates Platelet Activity by Inhibition of Platelet-mitochondrial Bioenergetics

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Cardiovascular diseases are the leading cause of death in the world. Platelets play a major role in cardiovascular events, binding to the damaged endothelium, activating, and forming thrombi. The development of drugs that prevent undesired platelet aggregation is of relevance. Using hydroquinone derivatives targeting mitochondria, we performed an extensive analysis to evaluate their antiplatelet effects on human platelets. First, we performed cytotoxicity studies and platelet aggregation screening with a series of compounds, being the one with the highest activity compound JP-I. JP-I does not present cytotoxicity and inhibits platelet aggregation by different agonists with IC_{50} values of 3.99 ± 1.43 μM for TRAP6; 7.16 ± 0.93 μM for PMA, and 36.33 ± 4.22 μM for arachidonic acid. Then, the mechanisms involved in JP-I antiplatelet activity were analyzed. The studies involved LC-MS/MS for quantitation of arachidonic acid-derived products; flow cytometry for detection of platelet activation markers, ROS production, mitochondrial membrane potential, and intracellular calcium levels; platelet spreading by fluorescence microscopy; intracellular ATP levels and ATP secretion by luminescence; and NADH in real-time by fluorimetry. Compound JP-I inhibited the oxidation of arachidonic acid by enzymatic pathways, by decreasing the levels of 12-HETE, 15-HETE, and TBX2. Moreover, JP-I reduced the expression of P-selectin, CD63, and PAC-1 in addition to platelet spreading. The mechanism seems to involve platelet mitochondria. Platelet oxygen consumption was lower in thrombin-activated platelets in the presence of JP-I. Compound JP-I diminished the oxidation of mitochondrial NADH and the electron transport during cellular respiration, exerts depolarization of the mitochondrial membrane in addition to increasing ROS levels. Importantly, we observed an increase in intracellular calcium levels while produced a decrease in ATP levels and secretion. In summary, our data indicate that compound JP-I decreases platelet activation by inhibiting mitochondrial bioenergetics. This work was supported by ANID/CONICYT, FONDECYT grants #1180427, CSIC grupos N° 536.

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