REDUCTION OF 1-CYS PEROXIREDOXINS BY ASCORBATE CHANGES THE THIOL-SPECIFIC ANTIOXIDANT PARADIGM, REVEALING A NEW ANTIOXIDANT FUNCTION OF VITAMIN C.

Monteiro, G.[1], Horta, B.B.[1], Pimenta, D.C.[2], Augusto, O.[3], Netto, L.E.S.[1]

[1]Depto de Genética e Biologia Evolutiva, Instituto de Biociências – USP; [2]Lab. Espectrometria de Massa, CAT/CEPID, Instituto Butantan; [3]Depto de Bioquímica, IQ-USP.

Peroxiredoxins (Prx) are widely distributed peroxidases that can be divided into 1-Cys and 2-Cys-Prx groups, based on the number of conserved cysteine residues that participate in their catalytical cycle. Prx have been described to be *strictly* dependent on thiols (RSH) but here we show that ascorbate (vitamin C) also reduces 1-Cys-Prx from several taxonomic groups although not 2-Cys-Prx. Reduction by ascorbate is partly related to the fact that the oxidized form of 1-Cys-Prx is a stable sulfenic acid (Cys-SOH) instead of a disulfide. In addition, a histidine residue in the active site is required. In fact, we engineered a 2-Cys-Prx with these two features and it displayed ascorbate peroxidase activity. The biological relevance of ascorbate reduction was suggested by the catalytic efficiency of the ascorbate-dependent activity estimated for Prdx6 from *Rattus norvegicus* ($k_{cat}/K_M \sim 0.9x10^6$ $M^{-1}.s^{-1}$), which is comparable to those previously reported for thiol-dependent processes. The peroxidase activity consistently decreased when ascorbate was depleted from homogenates, suggesting a major role for ascorbate in Prxd6 reduction. Ascorbate may be the long sought-after biological reductant of 1-Cys-Prx, which is unknown in most cases. The ascorbate/protein sulfenic acid pair represents a new aspect of redox biochemistry yet to be explored *in vivo*.