

Post-translation tyrosine phosphorylation switches cytochrome c dynamics.

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Beyond its role as an electron carrier, cytochrome c has emerged as a major hub of a wide network of interactions controlling cell fate and DNA damage response in plants and mammals [1, 2]. Several post-translation modifications of cytochrome c have been related to diverse pathological events standing out among them Tyrosine 48 phosphorylation.

The biophysical analysis of phosphorylated forms of the heme protein remains challenging. These species are unstable and sensitive to unspecific phosphatase enzymes. Further, in vitro phosphorylation assays await for the discovery of a kinase specific for cytochrome c. Hence, we replaced Tyr48 by the non-canonical amino-acid p-carboxy-methyl-L-phenylalanine.

Calorimetry and NMR Chemical Shift Perturbation analysis indicate that the phosphomimic mutation affects cytochrome c affinity towards its various binding sites on the electron transport chain, thereby modifying its diffusion pathway between respiratory complexes. It also enhances hemeprotein peroxidase and cardiolipin oxidation activities and hinders its ability to elicit caspase activation. Hence, we tackled the challenge of analyzing the structural features affected by this modification.

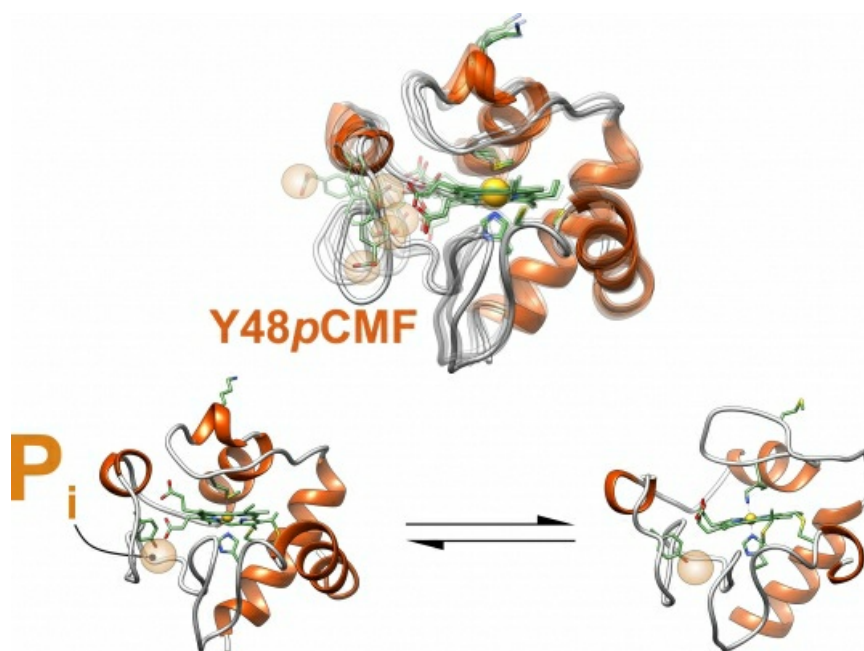
Despite its apparent simplicity, the globular structure of cytochrome c comprises five folding units with distinct stabilities. Four of them display pH dependent conformational changes. Among them stands out the so-called alkaline transition, involving the exchange of the sixth heme iron ligand. Notably, the phosphomimic mutation lowers the pKa value for this transition towards physiological pH values, as monitored by diverse spectroscopies [3].

Although the protein keeps the overall fold upon the modification, integrated principal component analysis of fluorescence and CD spectra indicates a destabilization of the folding unit which lodging the mutation. Accordingly, the analysis of NMR relaxation rates and heteronuclear NOEs indicate enhanced dynamics in this region and loops nearby. Additionally, XAS spectroscopy shows an enhancement of the heme-porphyrin dynamics on the reduced forms. Further NMR analysis allowed us to obtain the protein 3D structure and confirm the above findings, thereby providing a rationale to understand the pleiotropic effects that cytochrome c phosphorylation at Tyr48 bears on cell metabolism.

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[2] Gonzalez-Arzola, K. et al., (2015) Proc Natl Acad Sci U S A 112 (32), 9908-13.

[3] Guerra-Castellano, A. et al. (2015) Chem. Eur. J. , 15004-15012.



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