Title:	Catalytic Promiscuity of Cytochrome c towards Proton Transfer Reaction
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Keywords:	Biocatalysis Biocatalytic promiscuity Catalytic Promiscuity of Cytochrome Fluorescence Spectroscopy
Issue Date:	May-2020
Publisher:	IISER Mohali
Abstract:	In this study we have investigated the ability of cytochrome c (cyt c) as a catalyst for proton transfer reaction. Cyt c is small, globular heme protein having molecular weight ~12500. It is most primitive omnipresent protein which is present in all forms of aerobic organisms as well as in some anaerobic organisms. Here it is worthy to mention that the proteins/enzymes evolved in the early days of the evolutionary processes may possess wider range of substrate specificity to carry out multiple tasks as compared to the recently evolved enzymes. Apart from its primary function as an electron transporter in respiratory chain, cyt c is also known for its peroxidative property in lipid membrane by exposing heme moiety (its tertiary gets unfolded in membrane mimetic media) to the substrate. This now enables the peroxide substrate to bind with iron centre to show its peroxidase property. This made us curious to investigate the proton transfer ability of cyt c and fortunately, we have found that cyt c shows catalytic promiscuity towards proton transfer reaction, but this effect is strictly restricted to membrane mimetic media such as micelles and vesicles. Other enzymes/proteins like Lipase, Alkaline Phosphatase, Haemoglobin, HRP, catalase and lysozyme were also tested for proton transfer catalysis but none of them showed any catalytic rate. 5-nitrobenzisoxazole (NBI) was used as model substrate to study proton transfer by following Kemp elimination reaction. The catalytic rate by cyt c is found to increase with its residence at hydrophobic environment and also with the degree of unfolding of cyt c. The proximal histidine (His-18) moiety near hemin group is might be acting as base and responsible for the abstraction of proton from NBI (substrate) to form 2-CNP (product). Interestingly, In comparison with the aqueous buffer we have found approximately 250- fold increased KE catalysis by cyt c. As a whole, unprecedented catalytic promiscuity of cyt c towards proton transfer reaction has been found in this study, which can be highly significant in the evolutionary context, taking into consideration its role in delineating phylogenetic tree and also for producing biocatalyst with programmable multi-functional properties.
URI:	http://hdl.handle.net/123456789/1378 (http://hdl.handle.net/123456789/1378)

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