Title:	Superior Proton-Transfer Catalytic Promiscuity of Cytochrome c in Self-Organized Media
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Keywords:	Biocatalytic promiscuity Cytochrome c Kemp elimination Membrane-mimetic system
Issue Date:	2020
Publisher:	Wiley-VCH GmbH
Citation:	ChemBioChem, 2020(21), PP. 1-8.
Abstract:	Evolutionarily elderly proteins commonly feature greater catalytic promiscuity. Cytochrome c is among the first set of proteins in evolution to have known prospects in electron transport and peroxidative properties. Here, we report that cyt c is also a proficient proton-transfer catalyst and enhances the Kemp elimination (KE; model reaction to show proton transfer catalytic property) by ~750-fold on self-organized systems like micelles and vesicles. The self-organized systems mimic the mitochondrial environment in vitro for cyt c. Using an array of biophysical and biochemical mutational assays, both acid–base and redox mechanistic pathways have been explored. The histidine moiety close to hemin group (His18) is mainly responsible for proton abstraction to promote the concerted E2 pathway for KE catalysis when cyt c is in its oxidized form; this has also been confirmed by a H18A mutant of cyt c. However, the redox pathway is predominant under reducing conditions in the presence of dithiothreitol over the pH range 6–7.4.

Interestingly, we found almost 750-fold enhanced KE catalysis by cyt c compared to aqueous buffer. Overall, in addition to providing mechanistic insights, the data reveal an unprecedented catalytic property of cyt c that could be of high importance in an evolutionary perspective considering its role in delineating the phylogenic tree and also towards generating programmable designer biocatalysts.

URI: https://chemistry-europe.onlinelibrary.wiley.com/doi/10.1002/cbic.202000768
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