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HEXAAMMINERUTHENIUM AS AN ELECTRON DONOR TO MITOCHONDRIAL CYTOCHROME OXIDASE: MEMBRANE POTENTIAL GENERATION IN THE ABSENCE OF CYTOCHROME c

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SUMMARY. Cytochrome c oxidase can generate membrane potential in the absence of cytochrome c (e.g., in cytochrome c-deficient mitochondria or in proteoliposomes) with hexaammineruthenium as an artificial electron donor. Of several other redox mediators tested, phenazine methosulfate was found to be an efficient artificial substrate for membrane energization by cytochrome oxidase, whereas TMPD, DAD, DCPIP or ferrocyanide are virtually ineffective. The ability of $Ru(NH_3)_6^{2+}$ and phenazine methosulfate to support the generation of $\Delta\psi$ by cytochrome c-oxidase correlates with their effectiveness as electron donors to cytochrome a in the cyanide-inhibited membrane-bound enzyme.

INTRODUCTION

Mitochondrial cytochrome c-oxidase (ferrocytochrome c: oxygen oxidoreductase) is a terminal enzyme of the mitochondrial respiratory chain which catalyzes the reaction:

$$4\ c^{2+} + O_2 + 4H^+ \longrightarrow 4\ c^{3+} + 2H_2O$$

coupled to electric potential difference ($\Delta\psi$) and ΔpH generation across the coupling membrane [1].

For many years it has been considered that the enzyme is strictly specific for cytochrome c as an electron donor and for oxygen as an electron acceptor (for possible exceptions to this rule, see [2,3]). Indeed, although many artificial redox compounds (e.g., ferrocyanide, TMPD, halogen-substituted quinones) are able to reduce cytochrome oxidase metal redox centres, only a negligible continuous oxygen consumption can be maintained in their presence unless cytochrome c or sometimes other polycations [4-6] are added, which has been ascribed to a stimulating effect of the latter on the enzyme oxidation [6].

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Abbreviations: TMPD, N,N,N',N'-tetramethyl-p-phenylene diamine; DAD, diamino durene; DCPIP, dichlorophenolindophenol; $RuAm_6$, hexaammineruthenium; $\Delta\psi$, transmembrane electric potential difference; TPP^+, tetraphenylphosphonium.

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