Localization of a ferricyanide-reactive site of cytochrome b–c_1 complex, possibly of cytochrome b or ubisemiquinone, at the outer face of submitochondrial particles

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When succinate oxidation by submitochondrial particles is blocked by antimycin, NoHOQnO or funiculosin, addition of ferricyanide restores oxygen uptake coupled to membrane potential generation. The effect of ferricyanide is abolished by mucidin or myxothiazol, as well as by KCN. The data strongly favor a cyclic redox loop mechanism in site 2 and show that either heme of the ferrous cytochrome b or ubisemiquinone formed in the QH_2-oxidizing center of complex b–c_1 is accessible to ferricyanide at the outer (M) side of the submitochondrial particle membrane.

Respiratory chain *Q-cycle* *Cytochrome* b *Membrane potential* *Cytochrome* b–c_1 *site inhibitor* *Redox center topography*

1. INTRODUCTION

Ferricyanide has been widely used as a non-penetrating oxidant in studies of membrane-bound redox chain topography [1,2]. In intact mitochondria, electrons entering the respiratory chain via the internally localized dehydrogenases are accessible to ferricyanide only via the externally localized cytochromes c and c_1, so that reduction of $Fe(CN)_6^{3-}$ by succinate or NAD-dependent substrates is highly sensitive to antimycin [3–6].

In inverted submitochondrial particles (SMP), succinate dehydrogenase and NADH dehydrogenase are exposed at the outer (M) side of the membrane and react readily with ferricyanide [1,2,7].

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Abbreviations: NoHOQnO, 2-(*n*-nonyl)-4-hydroxyquinoline *N*-oxide; PCB , phenyldicarbaundecaborane anion; SMP, submitochondrial particles; $\Delta\psi$, transmembrane electric potential difference

Scattered evidence for reaction sites other than the dehydrogenases can be found in [8–12] but the identity of these sites remains obscure. The situation has been complicated by the presence of uninverted and ruptured particles in the conventional preparations of SMP in which ferricyanide could be reduced via cytochromes c and c_1 with low K_m.

It was found in [13–16] that addition of ferricyanide to SMP in the presence of succinate and antimycin restored membrane potential generation. This effect of ferricyanide was not observed under anaerobic conditions and was inhibited and prevented by cyanide. As only the coupled inside-out vesicles are seen by the PCB^- uptake method [17,18] used in [13,14], the observation pointed to the presence of a ferricyanide-reactive site of cytochrome b–c_1 complex, possibly of cytochrome b, on the M-side of the membrane.

Here we give further evidence that ferricyanide can drain electrons from the respiratory chain of SMP via some component(s) of cytochrome b–c_1 complex exposed at the outer face of the mem-

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