Microwave Hall Mobility Measurements on Heavy Beef Heart Mitochondria

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Abstract

The observed initial microwave Hall mobility values at 1.21 tesla of heavy beef heart mitochondria is at least six times greater than that observed for bovine serum albumin at similar resistivity values. The respiratory inhibitor cyanide significantly reduces the initial Hall mobility values for HBHM and for a preparation of HBHM cytochrome oxidase.

The four enzymic complexes of the respiratory chain were partially or completely separated. Of these complexes cytochrome oxidase exhibits the largest microwave Hall mobility.

The maximum hydration content of loosely bound water for freeze-dried preparations of cytochrome oxidase is 5% by weight; 60% of this hydration content is driven off by microwave power. Since the effective ac resistivity of the samples of cytochrome oxidase did not appreciably vary with changes in hydration content, the true resistivity of cytochrome oxidase has a value of the order $5 \times 10^3$ ohm cm and possibly much lower.

The electron transport pathway (as measured by Hall signal) of cytochrome oxidase is irreversibly damaged by prolonged exposure to microwave irradiation at 9.2 GHz. This is accompanied by the complete loss of capacity to oxidise ferrocytochrome c. Such changes do not occur with HBHM or with the other respiratory complexes.

There appears to be a direct relationship between observed Hall signals and the capacity of cytochrome oxidase to oxidize ferrocytochrome c. There is a "background" signal which is not directly related to electron transport but which is dependent on the conformation of the cytochrome oxidase.

The observed electronic parameters of cytochrome oxidase do not depend appreciably on its redox state.

Acid denaturation of cytochrome oxidase drastically reduces the Hall signal, to include almost complete removal of the "background" signal. It also more than doubles ac resistivity.

An electron tunnelling model is outlined.

Introduction

The mechanisms of charge transfer in the respiratory chain are not fully understood. The fact that charge transfer is greatly inhibited and

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possibly completely stopped at liquid nitrogen temperatures has been interpreted in favour of transport of electrons via mobile carriers (e.g. coenzyme Q, cytochrome c) rather than mechanisms based on resonance energy transfer or long range electron transport through conduction bands.1,2

The observed temperature variation does not, in fact, preclude the possibility that charge transfer occurs via conduction bands within individual molecular complexes of the cytochrome system. Transfer between complexes could involve a temperature activated tunnelling or hopping mechanism between the individual conduction band systems. Potential energy barriers could also exist within a particular cytochrome complex, the barrier shape and hence temperature variation of charge transfer being dependent on the molecular conformation.

In previous work3 it was shown that freeze dried preparations of rat liver mitochondria gave an N-type Hall signal, much greater than that obtained from bovine serum albumin at the same resistivity values. This signal was significantly reduced by cyanide, but not by rotenone or Antimycin A.

**Materials and Methods**

The microwave Hall mobility measurements described here have been obtained using a microwave system based on that described by Trukhan.4 A detailed account of the pertinent theory and experimental procedure has been given elsewhere.5

Preparations of heavy beef heart mitochondria (HBHM) were obtained as described by Smith.6 The enzymic activities of the electron transport chain were partially or completely resolved to give Complex I + III (NADH-cyt. c reductase7), Complex II (succinate-coenzyme Q reductase8) and Complex IV (cytochrome oxidase9).

NADH-cytochrome c reductase was assayed as described by Halefi and Rieske,7 in the absence of added phospholipid. Cytochrome oxidase was assayed as described by Wharton and Tzagoloff.10 Succinate-cyt. c reductase (Complex II + III) activity was assayed as described by King.11 Succinate-coenzyme Q reductase was not assayed.

No contamination of cytochrome oxidase by NADH-cyt. c reductase or succinate cyt. c reductase could be demonstrated. The NADH-cyt. c reductase was contaminated to less than 5% with cytochrome oxidase and less than 0.01% with succinate-cyt. c reductase.

Protein was determined by the method of Lowry, Rosebrough, Farr and Randall.12

Preparations of cytochrome oxidase were treated as follows: to 1 ml samples were added (a) 1 ml of aqueous KCN (final conc. 10⁻⁴ M); 0.2 ml of hydrogen peroxide (10 vols); sodium dithionite (1 mg); 1 ml of ferrocytochrome c (1%), produced as described by Wharton and