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Microwave Charge Carrier Hall Mobility Measurements on Cytochrome-Oxidase Prepared from Heavy Beef Heart Mitochondria

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The mechanisms of charge transfer in the respiratory electron transport chain are not fully understood. The fact that charge transfer is greatly inhibited and possibly completely stopped at liquid nitrogen temperatures has been interpreted in favour of the transport of electrons via mobile molecular carriers (e.g. coenzyme Q (CoQ), cytochrome C (cyt. c) rather than mechanisms based on resonance energy transfer or long range electron transport through conduction bands [1, 2].

This 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 individual complexes could involve a temperature activated tunnelling or hopping mechanism between the 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.

The microwave Hall mobility measurements described here have been obtained using a microwave system based on that described by Trukhan [3]. A detailed account of the pertinent theory and experimental procedure has been given elsewhere [4]. The essential feature of this technique is the employment of a bimodal cavity resonating at a frequency of around 9.15 GHz and the fact that intra- and inter-crystalline defects can be ignored.

Previous measurements [5] on freeze dried samples of rat liver mitochondria gave volume-corrected values of between 50 and 80 $cm^2/V/sec$ for the electron Hall mobility. The Hall signals were

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greatly reduced by the respiratory inhibitor cyanide, but were not significantly affected by rotenone or antimycin-A. The marked effect of cyanide together with the low mobility value obtained for a lipid extract from mitochondria suggested that electronic conduction through the electron transport chain was being observed.

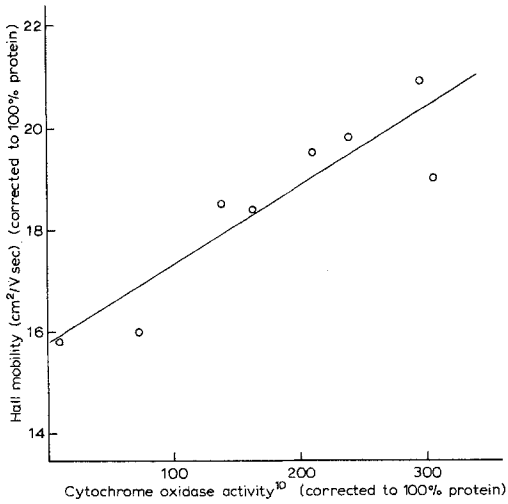
This work has been repeated with preparations of heavy beef heart mitochondria (HBHM) and similar results have been obtained, cyanide significantly reducing the observed microwave Hall signal. To ascertain which parts of the electron transport chain were responsible for the observed Hall signal, the constituent enzymic activities were partially or totally separated. Complexes I + III (NADH, cyt. c reductase) [6], II (succinate, CoQ reductase [7] and IV (cytochrome oxidase) [8] were isolated. Measurements were made in a dry nitrogen atmosphere and some typical results are shown in Table I.

TABLE I. Permittivity, resistivity and Hall mobility results obtained for various isolated complexes of heavy beef heart mitochondria

	ϵ^1	Resistivity ohm cm	Electron Hall mobility cm^2/V sec
Whole mitochondria	2.27	4.15×10^3	4.27
Complex I + III	2.46	2.54×10^3	0.54
Complex II	2.38	5.13×10^3	0.94
Complex IV	2.56	3.82×10^3	3.3

Of the isolated complexes, complex IV consistently gave the highest Hall mobility value, although the value never exceeded that obtained for the complete HBHM, indicating possibly that the electron transport pathway was disrupted during the isolation procedure. The Hall signals given by the other complexes were of the same order as those obtained for bovine plasma albumin [9] at similar resistivity values. Complex IV also differed from the remaining complexes and the complete HBHM in that the observed Hall signal decayed appreciably with time spent in the resonating bimodal cavity. The Hall mobility value of 4.27 cm^2/V sec for the complete HBHM was observed to fall to an apparently stable value of 4.03 cm^2/V sec during a period of 6 h spent in the cavity, whereas the value observed for complex IV fell from 3.3 to 1.97 cm^2/V sec over a period of some 24 h.

The reduction of the Hall mobility for complex IV with time spent in the cavity was observed to be accompanied by a reduction in the measured cytochrome oxidase activity (oxidation of ferrocytochrome c[10]). The results obtained for eight samples that had been exposed to the microwave power in the resonating cavity for varying periods of time are shown in Fig. 1. The protein content was determined for the samples,



and the results given in Fig. 1 were calculated for the case corresponding to the samples being 100% protein. This approach was taken since previous work [5] had shown that mitochondrial lipid exhibits a low microwave Hall signal, strongly indicating that the protein or lipoprotein component was responsible for the observed Hall signal.

From Fig. 1 there appears to be a definite relationship between the electron Hall mobility and the cytochrome oxidase activity. The least squares linear fit gives the relationship between the mobility (μ) and activity (A) as

$$\mu = 0.015\ A + 15.74$$

with a correlation coefficient of 0.90. The results indicate that there is a large "background" Hall signal not directly related to cytochrome oxidase activity and hence to electron transport in the respiratory electron transport pathway.

The observed reduction of Hall signal and cytochrome oxidase activity with time spent exposed to the microwave power ($\sim$60 mW) in the resonating cavity could have resulted from either oxidation or denaturation of the samples, or a combination of both processes. The resonating cavity was continuously flushed with dry nitrogen during the course of the measurements, hence oxidation effects were probably small and it was unlikely that the rate of oxidation of ferrocytochrome c by cytochrome oxidase was affected by alterations in the state of oxidation of cytochrome oxidase produced under these conditions.

The freeze drying of cytochrome oxidase does not appreciably alter its spectral or enzymic properties [11]. We have determined that 95% of

the loosely bound water content of cytochrome oxidase is removed by the freeze drying treatment, and that 60% of the remaining 5% loosely bound hydration content is removed on prolonged exposure to the microwave power in the resonating cavity. The concurrent loss of Hall signal and cytochrome oxidase activity may imply that the microwave power drives off that water necessary to preserve the haemprotein conformation essential to charge transfer and enzymic activity.

A direct investigation of the effects produced by denaturation was made by titrating a sample of the cytochrome oxidase to pH 1 with 6N-HCl in the presence of 0.5% Triton X-100. This treatment should have been sufficient to cause drastic alterations in the conformation of cytochrome oxidase. The observed Hall signal for the denatured sample was not only considerably lower compared with the initial signals observed for untreated samples but also some four times less than the "background" Hall signal (when calculated on a 100% protein basis). This would indicate that the electron conducting pathways not directly related to the respiratory chain, which may be responsible for the "background" Hall signal, were also deleteriously affected by acid denaturation.

That various conformational states of cytochrome oxidase may exist has recently been demonstrated [12]. Future microwave measurements will be directed towards investigating any possible relationships between the observed Hall signals and the related energy processing activities (degree of coupling to phosphorylation) for the various conformational states.

In conclusion we would suggest that these results indicate that future theories regarding the mechanism of charge transfer in the mitochondrial respiratory chain should include the possibility that within specific sections of the chain, charge transport occurs through physical solid-state type conduction bands. In itself this does not preclude the possibility that charge transport between the various complexes occurs via mobile molecular carriers or a mechanism involving molecular rotation. The complete charge transport process most likely involves a combination of various mechanisms, one of them being energy band conduction within a specific region of the cytochrome oxidase complex, at least for beef heart mitochondria.

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