CALCIUM–PROTON EXCHANGE IN CARDIAC AND LIVER MITOCHONDRIA

A. J. WILLIAMS and C. H. FRY
Cardiothoracic Institute, University of London, 2 Beaumont Street, London, W1N 2DX, England

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1. Introduction

The accumulation of Ca^{2+} by mitochondria in response to a respiration generated electrochemical gradient across the inner membrane has been the subject of a great deal of investigation (reviewed [1]). One aspect of mitochondrial Ca^{2+} transport that is the subject of much discussion is the charge-carrying capacity of Ca^{2+} entering the mitochondrion. It has been suggested [2] that each Ca^{2+} crossing the inner membrane effectively carries 1 positive charge, due to an obligatory calcium–phosphate symport mechanism with Ca_2^{4+}–HPO_4^{2-} as the translocated species. However, strong evidence has been provided [3] suggesting that Ca^{2+} transported into liver mitochondria, in response to the ejection of H^+ associated with electron transport, carry 2 positive charges/ion. They measured a H^+/Ca^{2+} ratio of 2 suggesting the maintenance of overall electroneutrality. These authors prevented phosphate movement by including *N*-ethylmaleimide in their reaction medium and used Ca^{2+}- and pH-sensitive electrodes to measure directly the relevant ion fluxes.

In the present investigation we have used a similar system, employing a Ca^{2+}-sensitive electrode, pH electrode and Clark-type O_2 electrode to monitor ion movements and O_2 utilisation in both liver and cardiac mitochondria.

A number of differences are known to exist between the Ca^{2+}-transporting systems of liver and cardiac mitochondria, such as differing sensitivities to magnesium inhibition and competition with ATP production [4]. However, the experiments described here suggest that the basic Ca^{2+}/H^+ stoichiometry of the two species of mitochondria is the same, both systems showing a H^+/Ca^{2+} ratio of 2.

2. Experimental

2.1. *Mitochondrial extraction*

New Zealand white rabbit cardiac and liver mitochondria were isolated by differential centrifugation. Hearts were homogenised using an Ultra turrex homogeniser in a medium containing 180 mM KCl, 10 mM EDTA and 0.5% bovine serum albumin (pH 7.2). The homogenate was centrifuged at 1000 × *g* for 5 min to remove contractile proteins and the supernatant recentrifuged at 9000 × *g* for 10 min. The mitochondrial pellet obtained in this way was resuspended in a medium containing 180 mM KCl and 0.5% bovine serum albumin and washed twice in this medium by centrifugation at 5000 × *g* for 10 min. The final pellet was resuspended in a medium containing 120 mM KCl and 3.0 mM Hepes, at pH 7.2.

Liver mitochondria were prepared by differential centrifugation following homogenisation using a Teflon homogenizer in 250 mM sucrose (pH 7.2). Again the final pellet was suspended in 120 mM KCl, 3.0 mM Hepes at pH 7.2. The protein concentrations of the suspensions were determined by the method in [5].

Experiments were performed at 25°C in a water-jacketed cuvette with total vol. 2.53 ml containing a Clark-type O_2 electrode (Rank bros. Bottisham, Cambridge). A glass pH electrode (401 M5 Ingold Gallenkamp) and Ca^{2+} electrode [6] were inserted through air-tight fittings. A common reference was used for the Ca^{2+} and pH electrodes and their outputs connected to the inputs of high input impedence operational amplifiers. The outputs of the amplifiers together with that of the O_2 electrode were recorded on a Watanabe pen recorder (multicorder MC 641)

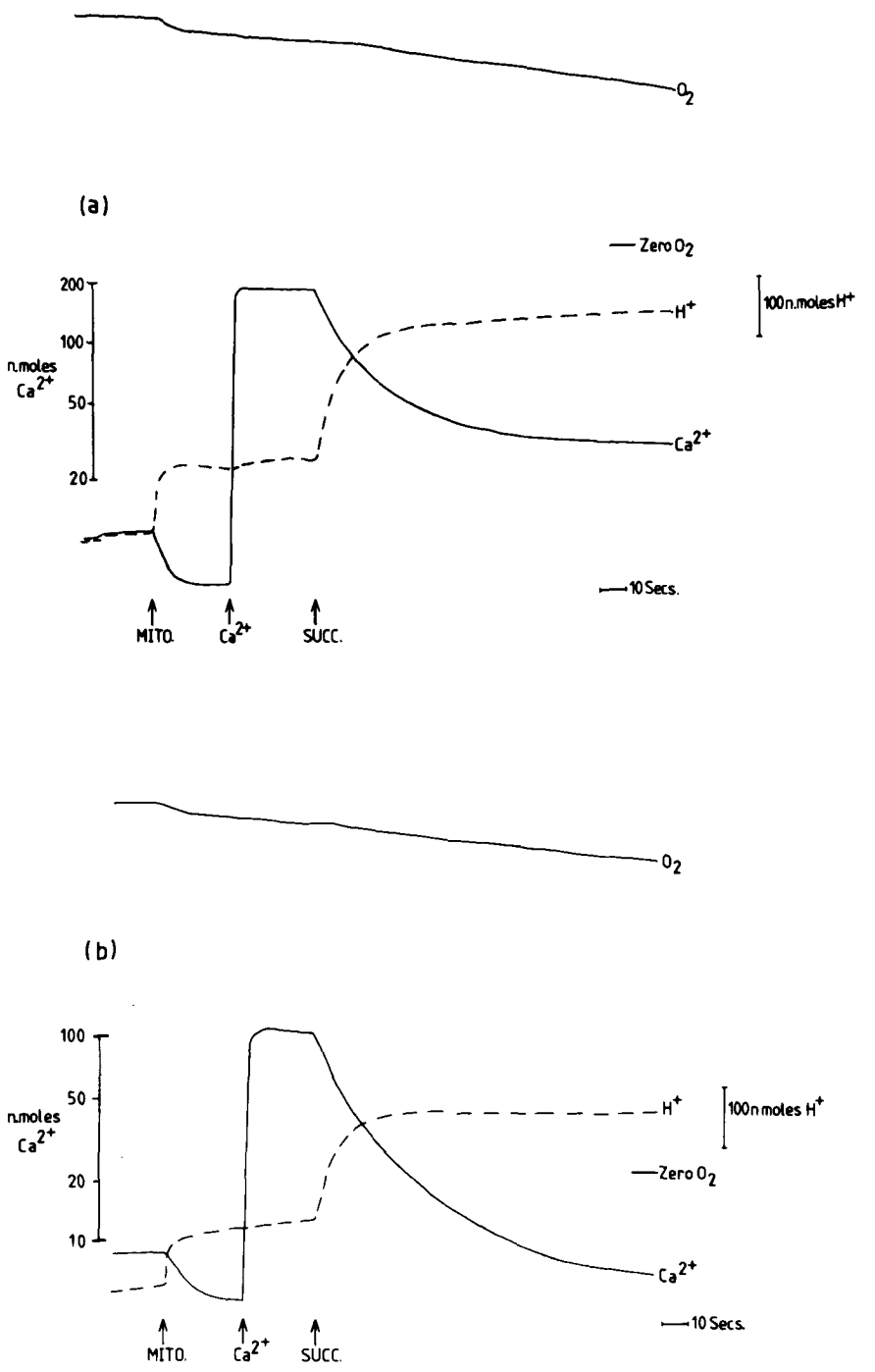

Fig.1. Experimental tracings. (a) Cardiac mitochondria: medium 120 mM KCl, 3 mM Hepes (pH 7.2), 2 μM rotenone, 40 nmol *N*-ethylmaleimide/mg protein; 74.0 μM initial Ca^{2+}; Ca^{2+} accumulation initiated by adding potassium succinate to give 2.4 mM final conc. (b) Liver mitochondria: experimental conditions as described for cardiac mitochondria; 42.0 μM initial Ca^{2+}.

and permanently stored on a Tandberg instrumentation tape recorder (series 115). Additions to the reaction medium were made through a small hole in the top of the cuvette.

The Ca^{2+} and pH electrodes were calibrated by adding known amounts of $CaCl_2$ and HCl. The response time of both these electrodes was <300 ms for a 95% deflection.

3. Results and discussion

The stoichiometric relationship of Ca^{2+} accumulation and H^+ ejection by liver and cardiac mitochondria was investigated in a medium containing 120 mM KCl and 3.0 mM Hepes, at pH 7.2, using potassium succinate as respiratory substrate. Records of typical experiments are shown in fig.1. As indicated, rotenone (final conc. 2 μM) and *N*-ethylmaleimide (40 nmol/mg protein) were added to the basic reaction medium. Mitochondria (4 mg protein) were added and the system allowed to equilibrate. In all experiments mitochondria immediately accumulated contaminant Ca^{2+} in the reaction medium, producing an associated acidification of the medium (fig.1a,b). Following this period of preincubation the Ca^{2+} content of the medium was adjusted by the addition of $CaCl_2$. Calcium accumulation was then initiated by adding 20 μl of a solution of potassium succinate to give final conc. 2.4 mM (fig.1). The addition of this respiratory substrate initiated an immediate removal of Ca^{2+} from the medium and an associated acidifica-

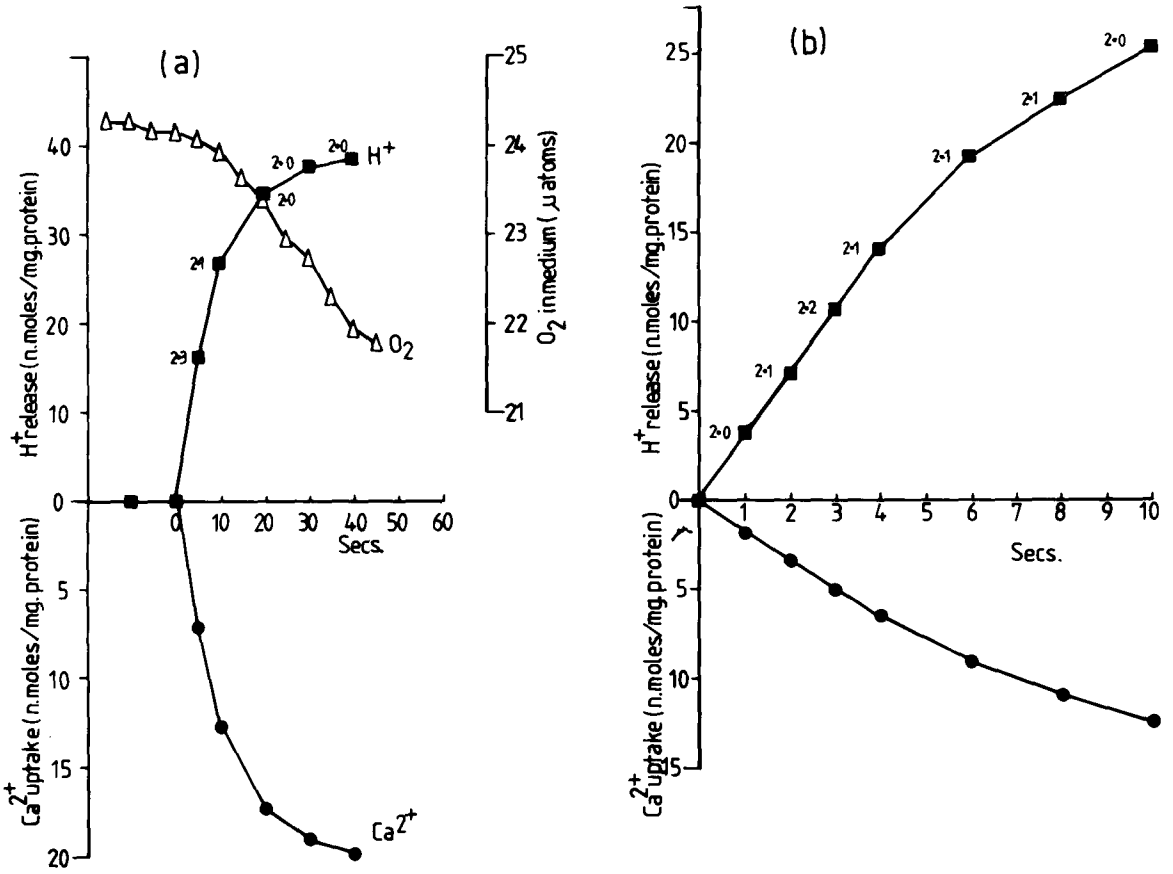

Fig.2. (a) Time course of Ca^{2+} accumulation, H^+ release and O_2 utilisation by cardiac mitochondria, experimental conditions as in fig.1; 35 μM initial Ca^{2+}. (b) Initial phase of Ca^{2+} accumulation and H^+ release. Figures along the traces are the H^+/Ca^{2+} ratios expressed as nmol H^+ released/mg protein to nmol Ca^{2+} accumulated/mg protein.

tion due to H^+ ejection. In addition, the O_2 utilisation of the mitochondria was increased.

Data from similar experiments are plotted in fig.2. To ease comparison with H^+ plots the logarithmic output of the Ca^{2+} electrode has been replotted on a linear scale. Figure 2a shows the time course of Ca^{2+} uptake, H^+ ejection and O_2 utilisation of cardiac mitochondria at 35 μM initial free Ca^{2+}, together with the H^+/Ca^{2+} ratios calculated as nmol H^+ released/mg protein to nmoles Ca^{2+} accumulated/mg protein, at various times during the course of the reaction.

Figure 2b depicts the initial phase of Ca^{2+} accumulation and H^+ ejection for the same experiment. At all times the molar ratio of H^+ ejection to Ca^{2+} uptake approximates to 2. At this Ca^{2+} concentration the rates of both Ca^{2+} uptake and H^+ ejection were linear over the first few seconds of the reaction, with initial rates of 106.8 nmol Ca^{2+} uptake/mg protein/min and 213.6 nmol H^+ release/mg protein/min.

Figures 3a,b show a similar experiment using liver mitochondria and 42 μM initial free Ca^{2+}. As with cardiac mitochondria the rates of Ca^{2+} uptake and H^+ ejection are linear over the first few seconds of the reaction, with initial rates of 87.0 nmol/mg/min Ca^{2+} uptake and 185.0 nmol/mg/min H^+ ejection. Again the H^+/Ca^{2+} approximates to 2 throughout the reaction

The findings presented in this investigation are in good agreement with those recently reported by Reynafarje and Lehninger [3] for rat liver mitochondria. Using both rabbit liver and cardiac mitochondria we have found a consistent stoichiometry of 2 H^+ ejected for each Ca^{2+} accumulated by the mitochondria using succinate as respiratory substrate, suggesting the maintenance of overall electroneutrality during Ca^{2+} accumulation in both organelles.

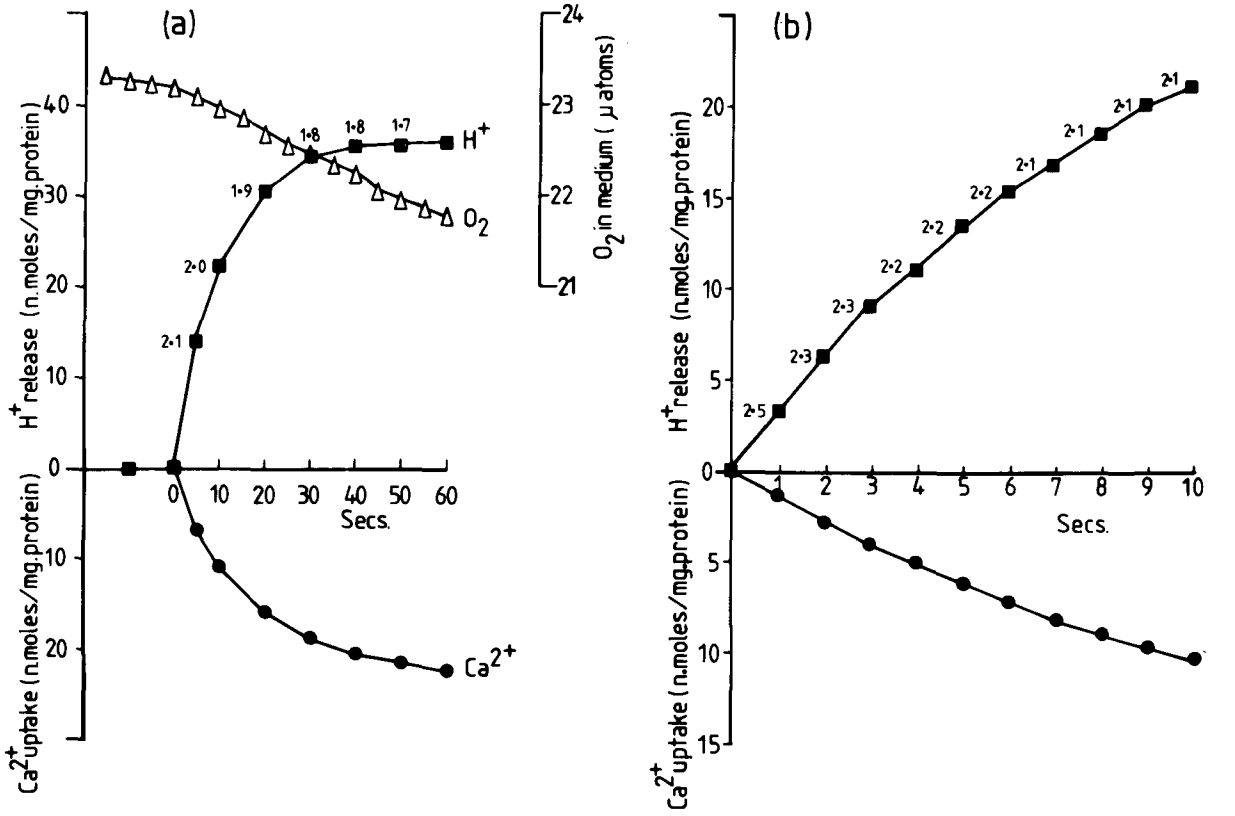

Fig.3. (a) Time course of Ca^{2+} accumulation, H^+ release and O_2 utilisation by liver mitochondria; 42 μM initial Ca^{2+}. (b) Initial phase of Ca^{2+} accumulation and H^+ release. As before figures along traces are the H^+/Ca^{2+} ratios.

Acknowledgements

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