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THE STOICHIOMETRY OF ION FLUXES DURING Sr^{2+}-INDUCED OSCILLATIONS IN MITOCHONDRIA

Y.V. EVTODIENKO, V.P. ZINCHENKO, E.L. HOLMUHAMEDOV, A.V. GYLKHANDANYAN and A.M. ZHABOTINSKY

Institute of Biological Physics, Academy of Sciences of the USSR, Pushchino and Institute of Biological Testing of Chemical Compounds, Kupavna, Moscow Region (U.S.S.R.)

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Summary

A quantitative study of H^+, K^+, Sr^{2+} and succinate fluxes in Sr^{2+}-induced oscillatory state of rat liver mitochondria is presented. It was shown that oscillation of succinate content in mitochondria occurs synchronously with oscillations of the cation fluxes. Total charge transferred across the membrane by the registered cations and the succinate-anion is equal to zero. Passive H^+-influx has been calculated at all stages of the oscillatory cycle. The conclusion is made that electroneutral 2 H^+/Sr^{2+} exchange is periodically induced in mitochondria. A value of $(2 \pm 0.2) \cdot 10^{-7}$ mol Sr^{2+}/min per mg protein. has been determined for Sr^{2+} by this type of exchange.

Introduction

Ion flux oscillation in the mitochondrial suspension has been the subject of extensive study [1—4]. It was revealed that the total charge transfer across the mitochondrial membrane during oscillation is not equal to zero [1,2]. The reason for this discrepancy was not elucidated. In the present work H^+, K^+, Sr^{2+} and succinate ion fluxes in mitochondria, and oxygen consumption rate during Sr^{2+}-induced oscillation [4] are measured and active and passive H^+ fluxes are calculated. It was shown that electroneutrality of the medium was maintained at all stages of the oscillatory cycle.

Materials and Methods

Rat liver mitochondria were isolated in solution containing 300 mM sucrose, 5 mM Tris-HCl and 0.5 mM Tris-EDTA, pH 7.5. Mitochondria were washed with the same medium omitting EDTA then suspended in sucrose-Tris-HCl medium and kept in ice. The activity of H^+, K^+ and Sr^{2+} was measured by ion-selective electrodes [5,6]. The response of bivalent cation electrode was considered Sr^{2+}-dependent only because the sensitivity of this electrode to Sr^{2+} was five times higher than to Ca^{2+}. The fast separation of mitochondria in the experiment with the [^{14}C]succinate was carried out by filtration of the suspension through the membrane filter 'Sinpore', 0.4 μm [7,8]. The time of separation was 10—15 s. The oxygen concentration in the medium was measured with a closed Clark-type electrode [9]. All measurements were done in an open temperature-controlled cell with continuous mixing.

Results

1. Determination of the total charge transfer across the mitochondrial membrane during oscillation

The changes of H^+, K^+ and Sr^{2+} concentrations in the external medium of mitochondrial suspension during oscillation are presented in Fig. 1 (curves a, b, c). The total charge transfer was determined from the curves presented. The calculated value of the positive charge change Σq^+ in the medium is presented as curve d in Fig. 1. It is seen from this curve that the oscillation of the positive

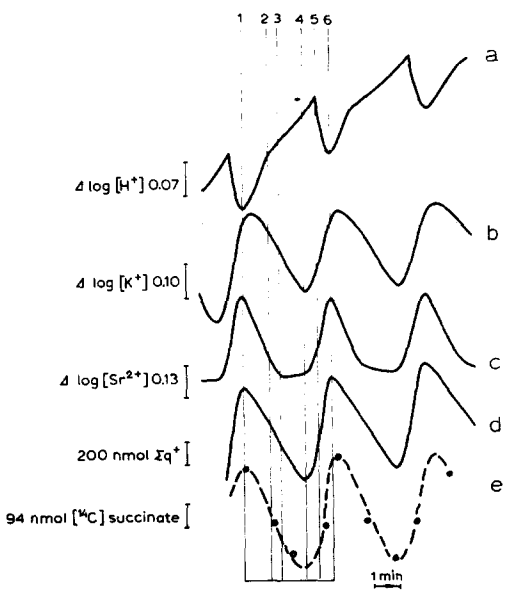

Fig. 1. Changes of H^+ (a), K^+ (b) and Sr^{2+} (c) concentration, total positive charge change Σq^+ (d) and succinate content (e) in the external medium of mitochondrial suspension during oscillations induced by addition of 600 nmol $SrCl_2$. Incubation medium: 20 mM sucrose, 5 mM succinate, 12 mM Tris-buffer, 1 mM KCl, 5 mg mitochondrial protein, 2.5 ng/mg rotenone, 7.5 ng/mg valinomycin. Final volume 2.0 ml, pH 7.5, 30°C.

charge in the medium occurs. Since the electroneutrality of the medium must be maintained, the transfer of some additional ionic species across the mitochondrial membrane might be proposed.

2. Determination of succinate content in mitochondria during oscillation

For clearing up the nature of ion species which compensate the total positive charge of the cation fluxes the succinate distribution in the oscillating mitochondrial suspension was studied. The changes of succinate contents in mitochondria during oscillation are presented in Fig. 1, curve e. This curve shows that the succinate motion through mitochondrial membrane occurs simultaneously with the positive charge transfer.

3. Estimation of active and passive H^+ fluxes during the oscillatory cycle

It should be noted that the registered changes of the external pH is the result of the two processes: the active H^+ transport from mitochondria by the respiration-dependent H^+ pump (H_a^+), and the passive motion of hydrogen ions into the mitochondria (H_p^+) (see Fig. 3). For estimation of the H^+ extrusion by H^+ pump of mitochondria (H_a^+), the rate of oxygen consumption by mitochondria during the oscillation was determined. As seen from Fig. 2b a cyclic change of oxygen concentration in the mitochondrial suspension occurs which depends on the rate of oxygen consumption by mitochondria and oxygen diffusion from the air. The rate of O_2 diffusion was determined after adding the inhibitor of the electron transport chain (CN^- or Antimycin A). The true value of the respiration rate was calculated by subtracting the rate of O_2 diffusion from the experimentally measured rate of oxygen concentration change into the mitochondrial suspension.

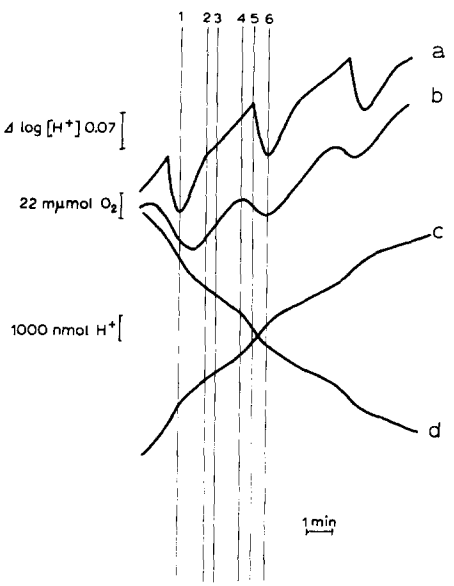

Fig. 2. Changes of H^+ (a), O_2 (b), H_a^+ (c) and H_p^+ (d) in the extramitochondrial medium during oscillations. Curves a (H^+) and b (O_2) are taken from the experiment presented in Fig. 1. Curves c (H_a^+) and d (H_p^+) were calculated (see text).

Assuming that H/O ratio for succinate oxidation is 4 [10], the production of hydrogen ions by the respiratory chain in the external medium was calculated (Fig. 2c). The comparative study of the registered H^+ concentration change (Fig. 2a) and of the theoretically calculated H^+ production (H_a^+) indicated the existence of the passive H^+ flux into the mitochondria (Fig. 2d) which was obtained by substracting the curve a from curve c.

4. Quantitative evaluation of ionic fluxes at different states of oscillatory cycle

In order to calculate the stoichiometry of ionic fluxes, the cycle of oscillation presented in Fig. 1 was divided according to the characteristic points (1—6). The absolute value of changes in extramitochondrial ion contents and their ratios at different states of the cycle are presented in Table I.

An active transport of Sr^{2+} and then K^+ into the mitochondria simultaneously with the H^+ efflux occurs at stages 1—2, 2—3 and 3—4 as a result of the proton-pump operation in the respiratory chain. As seen from Table I at stage 1—2 the ratio of the total charge ($2\ Sr^{2+} + K^+$) transferred into mitochondria to the effluxed hydrogen (H_a^+) equals one. The explanation for the deficiency of registered positive charge outside the mitochondria at stages 2—3, 3—4 and 4—5 is that the H^+ pump translocates more H^+ from the mitochondrial matrix than its registered amount in the external medium (Fig. 3, state I, II). The fact that $\Sigma q^+ \approx H_p^+ \approx 2$ succinate (Table I) indicates that H^+ returns into the mitochondria in protonated form of succinate [8,11—13,15]. At the stages

2—3 and 3—4 the ratio $\dfrac{2\ Sr^{2+} + K^+}{H_a^+}$

is less than one. This discrepancy might be due to the cation efflux as a result of action of H^+/Me^{2+} exchanger. Such exchange has been recently revealed [14]. Thus at the stages 2—3 and 3—4 H_p^+ influx is stipulated by succinate

TABLE I

ION FLUXES ACROSS THE MITOCHONDRIAL MEMBRANE AT DIFFERENT STAGES OF THE OSCILLATORY CYCLE

Arrows up indicate motion of ions from the mitochondria and arrows down motion of ions into the mitochondria. Experimental conditions as in Fig. 1. Suc, succinate.

Stages of oscillatory cycle	Registered changes of ion contents *				Calculated changes of ion contents *			Stoichiometry		
	H^+	K^+	Sr^{2+}	Suc **	Σ_q^+	H_a^+	H_p^+	$\frac{2suc}{\Sigma_q^+}$	$\frac{2Sr^{2+} + K^+}{H_a^+}$	$\frac{(2Sr^{2+} + K^+) + (H_p^+ - 2suc)}{H_a^+}$
1—2	75↑	36↓	196↓	169↓	353↓	426↑	351↓	0.99	1.004	0.974
2—3	17↑	118↓	42↓	88↓	185↓	380↑	363↓	1.02	0.531	1.026
3—4	36↑	236↓	0	108↓	200↓	556↑	520↓	1.10	0.424	0.971
4—5	25↑	100↑	69↑	88↑	263↑	440↑	415↓	0.72	0.541	1.086
5—6	77↓	314↑	150↑	267↑	537↑	414↑	491↓	1.00	—	—

* (equiv. of charge per mg protein) · 10^9.

** (molecules of [^{14}C]succinate per mg protein) · 10^9.

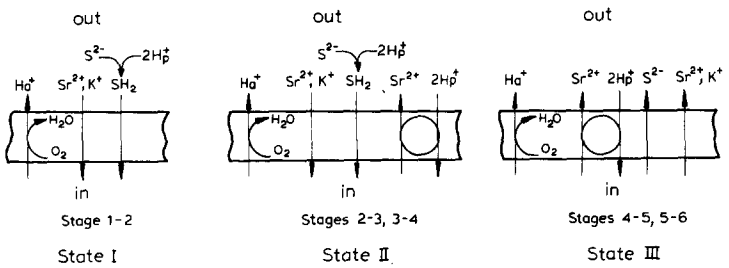

Fig. 3. The proposed scheme of ion transport across the mitochondrial membrane during oscillations. See text for explanation.

uptake and electroneutral H^+/Sr^{2+} exchange (see Fig. 3, state II). This assumption is supported by following (see Table I) equation:

$$\frac{(2\ Sr^{2+} + K^+) + (H_p^+ - 2\ \text{succinate})}{H_a^+} \cong 1$$

were $(2\ Sr^{2+} + K^+)$ is a registered cation influx and $(H_p^+ - 2$ succinate) is a amount of the Sr^{2+} effluxed from mitochondria by H^+/Sr^{2+} exchanger operation.

The stage 4—5 are characterized by unidirectional motion of the registered cations from mitochondria. This extrusion is accompanied by simultaneous efflux of succinate anions from the mitochondria, which can be seen from Table I (see also Fig. 3, state III). Subsequently, at the stage 5—6 the cations and succinate anions motion from mitochondria continues and an opposite H^+ influx appears. At the end of this stage the mitochondria return to the initial state and the cycle of the oscillation is repeated.

Thus the quantitative analysis of cation fluxes oscillation in mitochondrial suspension indicates appearance of the H^+/Me^{2+} exchanger in the mitochondrial membrane at the some stages of oscillatory cycle. Changes of succinate (or its derivatives) contents in mitochondria during oscillatory state shows a participation of protonated and ionized forms of succinate in oscillations.

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