INTRAMITOCHONDRIAL PHOSPHATE IS THE SOURCE OF PROTONS IN THE RESPONSE OF LIVER MITOCHONDRIA TO CATIONS

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1. Introduction

We have reported that after inhibition of phosphate transport in mitochondria, their respiratory response to divalent cations (Ca^{2+}, Sr^{2+}) was proportional to the intramitochondrial phosphate content [1]. A similar conclusion has been reached in [2]. We suggested that intramitochondrial phosphate was the source of protons for the proton pump linked to the respiratory chain.

The interpretation of these results raises two further questions:

1. Is the dependence of the respiratory response on intramitochondrial phosphate specific for divalent cations which have a specific porter system in mitodria? The importance of this question was stressed by the suggestion [3,4] concerning a mersalyl-insensitive phosphate transport linked to uptake of divalent cations.
2. Is there experimental support for the suggested relationship between intramitochondrial phosphate and proton ejection?

We have found that the temporary stimulation of respiration following the addition of K^+ plus valinomycin was parallel to the intramitochondrial phosphate content when the latter was changed experimentally. A similar parallelism between intramitochondrial phosphate and proton ejection was also present. This supports the view that intramitochondrial phosphate is the proton donor to the respiratory chain-linked proton pump after phosphate transport inhibition in mitochondria.

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2. Methods

Preparation of rat liver mitochondria, measurement of respiration, H^+ ejection and intramitochondrial phosphate content were as in [1,5]. Mitochondria were depleted of their phosphate content after mersalyl treatment by addition of ADP followed by oligomycin; phosphate uptake was induced by aerobic incubation prior to addition of mersalyl. Mersalyl at 25 nmol/mg mitochondrial protein was added to inhibit phosphate transport. 'Extra' oxygen consumption and H^+ ejection (in separate experiments) were triggered by addition of valinomycin and KCl or strontium nitrate to respiring mitochondria oxidizing succinate in presence of rotenone. The 'extra' oxygen consumption was calculated similarly to that in [6]. H^+ ejection was calculated on basis of the final pH change recorded and calibration by known amount of standard HCl.

3. Results

3.1. *Respiratory changes caused by K^+ plus valinomycin after inhibition of phosphate transport by mersalyl*

Addition of K^+ plus valinomycin to mitochondria having an intact phosphate transport system, and in presence of inorganic phosphate, results in release of respiration which lasts as long as oxygen is available [7]. In contrast with this, addition of K^+ plus valinomycin to mitochondria whose phosphate transport system was inhibited, caused only a short-lasting release of respiration (burst); after the stimulated period the respiration returned to almost the initial,

Table 1
'Extra' oxygen consumption caused by addition of either KCl plus valinomycin or of $Sr(NO_3)_2$ to mitochondria after changing their intramitochondrial phosphate content

	Sequence of additions	P_i nmol/mg protein	'Extra' oxygen consumption ng atom oxygen/mg protein elicited by	
			K^+ (+ val)	Sr^{2+}
Exp. 1	Valinomcyin, mersalyl, succinate, P_i	19.2	2.9	5.0
	Mersalyl, succinate, P_i	22.0	3.2	7.9
	Succinate, P_i, mersalyl	33.0	4.7	10.8
	Succinate, P_i, mersalyl, ADP, oligomycin	20.3	3.6	6.5
Exp. 2	Mersalyl, succinate, P_i, ADP, oligomycin	21.2	2.7	7.0
	Mersalyl, succinate, P_i	28.5	3.5	11.3
	Succinate, P_i, mersalyl	37.0	5.0	13.2
	Succinate, P_i, mersalyl, ADP, oligomycin	28.0	3.9	7.7

Mitochondria (2.6 mg protein/ml) were incubated at 25°C. Additions: 2.1 mM Tris–phosphate, 5.3 mM Tris–succinate, 25 nmol mersalyl/mg protein, 41.6 nmol ADP/mg protein, 2.1 μg oligomycin/mg protein, 83 ng valinomycin (val)/mg protein, 83 nmol strontium nitrate/mg protein and 4.5 mM KCl

prestimulation level. No subsequent stimulation could be elicited by addition of Ca^{2+} or Sr^{2+}. Restoration of phosphate transport by thiol compounds or addition of uncouplers was, however, effective in releasing respiration once more.

By variation of the intramitochondrial phosphate content it was found that the extent of the burst of respiration was proportional to the phosphate content of the mitochondria (table 1). Using the data of several experiments the regression equation calculated was: $y = 0.1\,x + 0.9$ (data of 19 determinations).

It is also seen from table 1 that at identical phosphate contents the stimulation of respiration by added Sr^{2+} was always considerably larger than the stimulation by K^+ plus valinomycin. This was also reflected in the regression equation calculated from the data of 'extra' respiration given by Sr^{2+}: $y = 0.37\,x - 1.4$ (see also [1]).

3.2. *Proton ejection from mitochondria after inhibition of phosphate transport*

If intramitochondrial phosphate is indeed the source of H^+ for the respiratory chain-linked proton pump, then the H^+ ejection which follows the addition of 'permeable' cations (Ca^{2+}, Sr^{2+} or K^+ together with valinomycin) to mitochondria with inhibited phosphate transport should be proportional to their phosphate content. Such a correlation was found to exist when either K^+ plus valinomycin or Sr^{2+} triggered proton ejection (fig.1).

Similarly to the stimulation of respiration, at identical intramitochondrial phosphate content, Sr^{2+} elicited always a larger proton ejection than K^+ plus valinomycin. The slopes of the 2 regression equations for the 2 cations did not differ so much

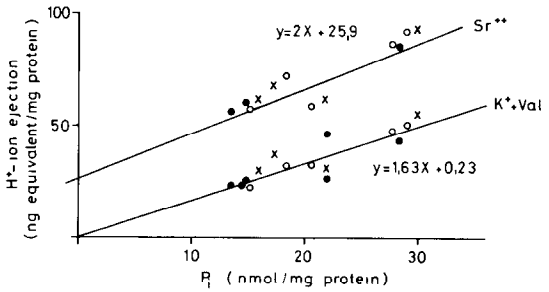

Fig.1. The relationship between H^+ ejection and phosphate content of mitochondria. H^+ ejection was elicited by addition of either 83 nmol strontium nitrate/mg protein, or of 4.5 mM KCl followed by 83 ng valinomycin/mg protein to mitochondria in which P_i transport was prior inhibited by addition of 25 nmol mersalyl/mg mitochondrial protein. The P_i content of mitochondria was changed as in table 1 [1]. The different symbols (○,●,×) refer to different experimental runs.

than the slopes calculated for respiration (see section 3.1.). The regression line of the data for Sr^{2+} intercepted the ordinate at the value of about 26 ng equiv. H^+ ejected/mg protein indicating that Sr^{2+} elicited significant proton ejection even if no intramitochondrial phosphate was present. The nature of the proton donor in the absence of intramitochondrial phosphate is as yet unknown.

4. Discussion and conclusions

Apparently the correlation between stimulation of respiration by divalent cations and intramitochondrial phosphate content found [1] also applies to monovalent cations provided a suitable ionophore, e.g., valinomycin is present. It was proposed [4] that stimulation of respiration by calcium (or strontium) in the presence of phosphate transport inhibitors is the consequence of calcium–phosphate symport through the calcium porter. This proposed pathway of phosphate transport does not function when the 'permeating' cation is potassium in the presence of valinomycin. Stimulation of respiration by cations can therefore occur independently of the proposed symport mechanism.

The correlation between intramitochondrial phosphate and proton ejection strongly suggests that intramitochondrial phosphate is in equilibrium with the proton pump of the respiratory chain and can act as a proton donor for this latter. The deprotonated phosphate ion is probably an important factor in the generation of intramitochondrial negativity which latter causes electrophoretic influx of 'permeable' cations. It also seems probable that in the absence of 'permeable' cations, the transmembrane potential gradient, negative inside, opposes effectively the proton pump, and in absence of proton ejection the respiration has to be slow (state 4). If 'permeable' cations are present, their electrophoretic movement neutralizes the transmembrane potential gradient, the proton pump can operate and respiration is released. This released respiration requires however the continuous supply of proton donor, which is in most cases phosphate. If phosphate transport is inhibited, both release of respiration and proton ejection are limited by the available intramitochondrial phosphate content.

There is no satisfactory explanation yet for the discrepancy between the responses to addition of Sr^{2+} and of K^+ in the presence of valinomycin. The possible mechanism is currently under investigation.

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