Ion transport in rat liver mitochondria: the effect of the incubation medium osmolarity

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A decrease in the incubation medium osmolarity from 320 to 120 mosM reverses the pH dependence of K^+ efflux from rat liver mitochondria. The K^+ efflux is no longer inhibited by oligomycin and a free radical scavenger butylhydroxytoluene. At 320 mosM, the addition of carbonyl cyanide 3-chlorophenylhydrazone (CCCP) accelerates the K^+ efflux, while EGTA inhibits it. At 120 mosM these CCCP and EGTA effects are reversed. In either case the K^+ efflux is inhibited by Mg^{2+}. The decrease in osmolarity changes the ruthenium red-insensitive Ca^{2+} efflux in the same manner. It has thus been shown that the modification of the mitochondrial structure by changing the incubation medium osmolarity results in a qualitative alteration of the systems regulating the K^+ and Ca^{2+} effluxes.

Mitochondria *Ion transport* *ATPase* *Osmolarity* *Free radical reaction*

1. INTRODUCTION

It has been reliably established that a change in energy state of mitochondria is associated with modification of their structure [1–4] and ion transport [5–7].

As shown in other studies, changing the osmolarity of the incubation medium alters the mitochondrial structure [1,16–19]. On the other hand, a correlation has been established between the mitochondrial structure modification and changes in the energy coupling parameters [8–11], and also in ion transport [6,12–15].

We demonstrated in [11] that alteration in the mitochondrial structure in the osmolarity range 100–500 mosM correlates with a qualitative change in the coupling mechanism of electron transport and ATP synthesis. We also found a connection between cation transport and ATP-synthetase [20,21]. Here we have investigated the effect of osmolarity (in the range 120–320 mosM) on the properties of K^+ and the ruthenium red-insensitive Ca^{2+} effluxes. The interaction of these fluxes with the operation of the ATP-synthetase complex was also studied.

2. MATERIALS IN METHODS

Rat liver mitochondria were isolated by differential centrifugation in a medium containing 250 mM sucrose, 5 mM Hepes 250 μM EDTA, pH 7.4 [22]. The final washing was performed in the same medium, but without EDTA. Protein in the mitochondrial suspension was assayed by the biuret method with bovine serum albumin as a standard [23]. K^+ in the incubation medium was measured with a potassium-selective glass electrode. The mitochondrial respiration was measured with a Clark oxygen electrode. Ca^{2+} movements were monitored spectrophotometrically on an Aminco DW-2 using arsenazo III as an indicator at 675 nm vs 685 nm. Mitochondria (1 mg

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Abbreviations: BHT, butylhydroxytoluene; CCCP, carbonyl cyanide-3-chlorophenylhydrazone; Mes; 2-(*N*-morpholine)ethanesulfonic acid

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protein/ml) were incubated in a medium containing 10 mM Mes, 10 mM succinate, 10 mM H_3PO_4, 30 μM $CaCl_2$, 2 μM rotenone. The pH of the medium was brought to the required value with Tris buffer. The required osmolarity was achieved by supplementing a corresponding amount of sucrose. 40 μM arsenazo III was added to the medium to assay Ca^{2+}.

3. RESULTS

A change in the incubation medium osmolarity within 120–320 mosM results in a drastic change of the K^+ efflux pH dependence. Fig.1 shows the pH dependence of K^+ efflux at 320 and 120 mosM. At 320 mosM, the K^+ efflux is decelerated on pH being increased from 6.7 to 7.6. The rate increases in the pH 7.6–7.9 interval. An osmolarity decrease to 120 mosM results in a qualitative change of the pH dependence of the K^+ efflux rate; under these conditions, a rate increase is registered only if the pH of the incubation medium is increased. A lowering of pH from 7.9 to 6.7 completely suppresses the K^+ efflux.

Fig.2 shows the K^+ efflux at two pH values (7.9 and 6.7) vs osmolarity. At pH 6.7, a gradual decrease in tonicity osmolarity from 320 to 120 mosM inhibits the K^+ efflux. Conversely, at pH 7.9 the maximal rate of the K^+ efflux is registered at low osmolarity, and it decreases with osmolarity increase.

We demonstrated previously that the induction

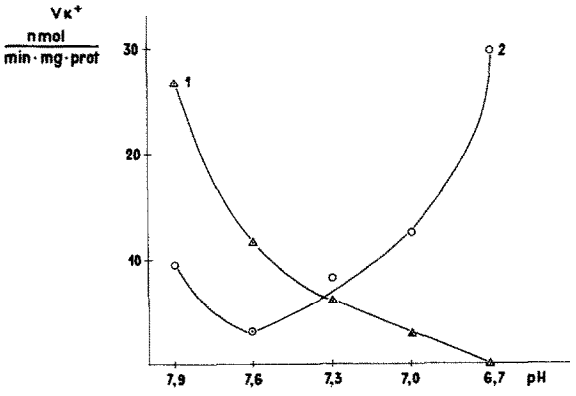

Fig.1. pH dependence of the initial rate of K^+ efflux from rat liver mitochondria at 120 (1) and 320 (2) mosM. Composition of medium: see section 2.

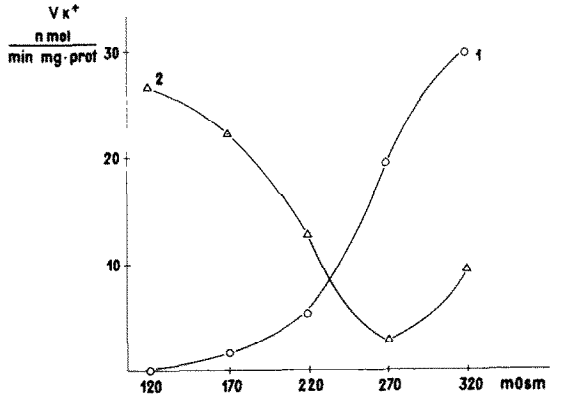

Fig.2. The initial rate of K^+ efflux from rat liver mitochondria at pH 6.7 (1) and 7.9 (2) vs osmolarity of the incubation medium. Composition of medium: see section 2.

of K^+ efflux from mitochondria at low pH values and high tonicity is suppressed by BHT, an inhibitor of free-radical reactions, and by a Ca^{2+} chelator, EGTA [20]. As seen in fig.4 (curve 4), BHT does not inhibit the K^+ efflux induced at 120 mosM and pH 7.9, whereas at 320 mosM and pH 6.7, a strong inhibition of the K^+efflux is observed

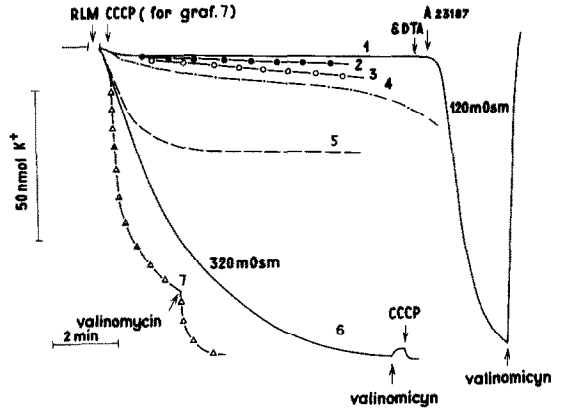

Fig.3. The sensitivity of K^+ efflux in rat liver mitochondria to inhibitors at 320 mosM and pH 6.7. Composition of medium: see section 2 (6). Control, (1) osmolarity of incubation medium decreased to 120 mosM. Incubation medium supplemented: (2) 10^{-4} M EGTA, (3) 5×10^{-3} M $MgCl_2$, (4) 5×10^{-5} M BHT, (5) 2 μg/mg protein oligomycin, (7) 1.5×10^{-6} M CCCP; Arrows indicate additions: 2×10^{-7} M valinomycin, 10^{-4} M EDTA, 10^{-6} M A23187.

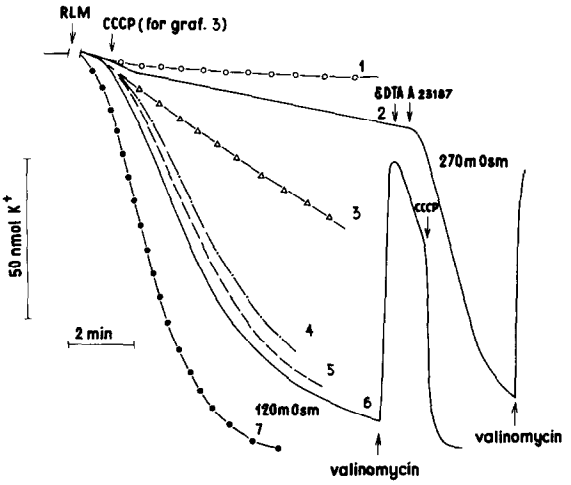

Fig.4. The sensitivity of K^+ efflux in rat liver mitochondria to inhibitors at 120 mosM, pH 7.9. Composition of medium: see section 2 (6). Control, (2) osmolarity of incubation medium increased to 270 mosM. Incubation medium supplemented: (1) 5×10^{-3} M $MgCl_2$, (3) 1.5×10^{-6} M CCCP, (4) 5×10^{-5} M BHT, (5) 2 μg/mg protein oligomycin, (7) 10^{-4} M EGTA. Arrows indicate additions: 2×10^{-7} M valinomycin, 10^{-4} M EDTA, 10^{-6} M A23187.

(fig.3, curve 4). If the osmolarity is lowered from 320 to 120 mosM, the K^+ transport system ceases to be activated by Ca^{2+}. At 120 mosM, addition of EGTA does not inhibit the K^+ efflux the way it does at 320 mosM (fig.3, curve 2), but reduces the lag period of K^+ efflux induction (fig.4, curve 7). Under these conditions low Ca^{2+} concentrations inhibit the K^+ efflux. It follows from the above data that a change in the incubation medium osmolarity leads to a change in K^+ efflux parameters. We also showed that the coupling of K^+ efflux to the mitochondrial energy state is changed simultaneously. According to [20,21,25,26], the induction of K^+ efflux in an isotonic medium and at acidic pH is accompanied by an increase in the respiration rate and by a fall in the mitochondrial transmembrane potential. This attests to electrogenic ion fluxes appearing in the system. Correspondingly, as seen in fig.3 (curve 6), under these conditions the K^+ flux is not reversed by adding valinomycin upon termination of the K^+ efflux. A K^+ flux reversal is observed only at the initial stages of K^+ efflux [24].

The K^+ efflux at 120 mosM and pH 7.9 is not accompanied by any significant respiration rate increase; accordingly, the K^+ flux is reversed upon valinomycin addition after the completion of the K^+ efflux from mitochondria (fig.4, curve 6). These data show that at low tonicity the K^+ efflux is largely non-electrogenic. We demonstrated that the value of electrogenic fluxes falls off sharply at 120 mosM and pH 6.7, compared with 320 mosM. A similar result was obtained with KCl as osmotic support. This demonstrates that electrogenic leakage changes are the effect of osmolarity.

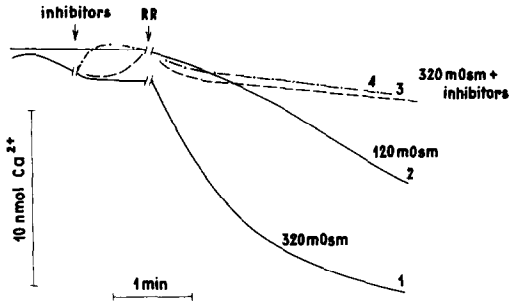

Fig.5. The effect of oligomycin and BHT on the ruthenium red-insensitive Ca^{2+} efflux, at 320 mosM, pH 6.7. Composition of incubation medium: see section 2 (1) Control, (2) osmolarity of incubation medium decreased to 120 mosM. Medium supplemented: (3) 2 μg/mg oligomycin, (4) 5×10^{-5} M BHT. Arrow indicates the addition: 3 nmol/mg protein of ruthenium red, 2 min after the addition of mitochondria.

At 320 mosM, the oxidative phosphorylation

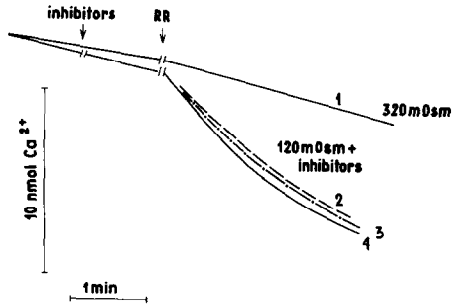

Fig.6. The effect of oligomycin and BHT on the ruthenium red-insensitive Ca^{2+} efflux at 120 mosM, pH 7.9. Composition of medium: see section 2. (4) Control, (1) osmolarity of incubation medium increased to 320 mosM. Incubation medium supplemented with: (2) 2 μg/mg oligomycin, (3) 5×10^{-5} M BHT. Arrow indicates the addition: 3 nmol/mg protein of ruthenium red 2 min after the introduction of mitochondria.

uncoupler CCCP increases the K^+ efflux rate (fig.3, curve 7), but decrease it at 120 mosM (fig.4, curve 3). As seen from figs. 5 and 6, a change in the incubation medium osmolarity affects not only K^+, but also Ca^{2+} transport. An osmolarity decrease from 320 to 120 mosM is accompanied by a reversal of the pH dependence of the ruthenium red-insensitive Ca^{2+} efflux (figs.5,6). At 320 mosM and pH 6.7, BHT inhibits Ca^{2+} efflux (fig.5, curve 4), but does not do so at 120 mosM and pH 7.9 (fig.6, curve 3).

The induction of K^+ efflux (fig.3, curve 5) and of the ruthenium red-insensitive Ca^{2+} efflux (fig.5, curve 3) in an isotonic medium and at acidic pH is inhibited by oligomycin. Yet, at 120 mosM and pH 7.9, oligomycin ceases to suppress the induction of these fluxes (fig.4, curve 5; fig.6, curve 2).

4. DISCUSSION

A natural question arises as one considers the effect of tonicity on the pH dependence of K^+ efflux from mitochondria: which stage of the process is changed with the change in tonicity? It is known in particular that activation of K^+ efflux in mitochondria correlates with the Mg^{2+} concentration in the matrix [14,15,25–28]. We demonstrated that the osmolarity and pH of the incubation medium control Mg^{2+} transport. According to the data in figs.3 and 4 only a weak K^+ efflux from mitochondria takes place at 270 mosM (pH 7.9) and 120 mosM (pH 6.7). Under these conditions, the addition of the A23187 carrier and EDTA resulting in Mg^{2+} efflux from the matrix induces K^+ efflux as well. The results of these experiments explain the inhibition of K^+ efflux by Mg^{2+} (fig.3, curve 3; fig.4, curve 1).

Comparison of the effect of tonicity on K^+ and the ruthenium red-insensitive Ca^{2+} transport in mitochondria reveals a very close similarity of the regulatory systems. Indeed, it is evident from our data that the tonicity of a medium has the same effect on the sensitivity of the fluxes of these ions to various inhibitors and pH changes in the incubation medium (figs 3–6). The data in [21] (that K^+ and Ca^{2+} fluxes are equally suppressed by respiratory chain inhibitors) likewise support our conclusion. It may be presumed therefore that the K^+ transport system and that of Ca^{2+}, insensitive to ruthenium red, either obey the same regulation pattern or are one and the same system [29]. Consequently, a change in the incubation medium tonicity leads to a qualitative change of ion transport parameters. Of special significance is the fact that at high tonicity K^+ and Ca^{2+} transport is controlled by ATP-synthetase – the oligomycin inhibition of ATP synthetase results in the inhibition of the transport of these ions.

Thus, according to [11], tonicity also affects the nature of ATP-synthetase interaction with the respiratory chain in oxidative phosphorylation.

At high tonicity (500 mosM), ATP-synthetase and the respiratory chain behave as autonomous systems, and at 100 mosM, they operate as a single functional complex as far as their kinetic parameters are concerned. The effect of tonicity and consequently, of the structural state of mitochondria [1,16–19] on the properties of the two essential mitochondrial systems – oxidative phosphorylation and endogenic ion transport – supports our suggestion that mitochondria may be in two qualitatively different functional states.

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