ON THE MECHANISM OF ENERGY-DEPENDENT CONTRACTION OF SWOLLEN MITOCHONDRIA

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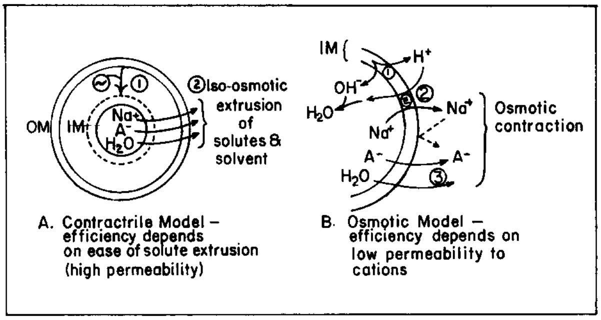
Summary - Electrophoretic cation permeability, as estimated by rates of passive swelling of mitochondria suspended in Na+ and K+ nitrate, increases with increasing temperature and elevated pH and is inhibited by Mg^{+2}. Mitochondria swollen in Na+ nitrate at 37° and pH 8.2 contract in an energy-dependent reaction. The efficiency of the contraction (absorbance change per O_2 or ATP consumed) decreases with increased electrophoretic cation permeability as established by either elevated pH or addition of gramicidin. Efficiency is increased by Mg^{+2}. This inverse relationship between electrophoretic cation permeability and efficiency of contraction is compatible with an osmotic contractile mechanism which depends on the Na+/H+ exchanger present in the mitochondrial membrane.

INTRODUCTION

Numerous examples have been reported in which swollen mitochondria utilize the energy of substrate oxidation or exogenous ATP to contract and extrude accumulated ions (see Refs 1-3 for reviews). Such contraction reactions have been explained in terms of either (a) extrusion of water and solutes due to a contractile change, or (b) an ion-extruding mechanism which produces an osmotic contraction (2,4,5). The features of these two models are shown in the diagrams in Figure 1. The contractile model (Fig. 1A) requires a high permeability to cations for efficient operation whereas the opposite holds true for the osmotic model (Fig. 1B). The early observations of Azzi and Azzone (4) clearly suggested that low cation permeability was associated with the contraction reaction, but more recent estimates of the pore radius of swollen mitochondria by Massari and Azzone (6) suggested a high permeability which would be incompatible with the osmotic model. In the present communication we wish to summarize a number of recent experiments which indicate that a low transmembrane, electrophoretic permeability to cations is essential for efficient energy-dependent contraction.



A. Contractrile Model – efficiency depends on ease of solute extrusion (high permeability)

B. Osmotic Model – efficiency depends on low permeability to cations

Fig 1 - Comparison of contractile model (A) with osmotic model (B) for energy-dependent contraction of swollen mitochondria.

RESULTS

Isolated beef heart mitochondria suspended in 100mM K+ or Na+ nitrate at neutral pH do not swell unless the membrane is made permeable to the cation by addition of gramicidin (or by some other membrane modification) (7). Since much evidence suggests that the membrane is permeable to nitrate under these conditions (7,8) and that nitrate diffusion can create a negative interior potential (8), it appears that swelling is prevented by a low electrophoretic permeability to monovalent cations. We have recently noted that much more swelling is obtained when mitochondria are incubated at pH8-8.5 in K+ or Na+ nitrate. In addition, all mitochondrial swelling reactions tested show an enhanced temperature sensitivity above about 27°, so that when heart mitochondria are incubated in 100mM Na+ nitrate at pH 8.2 and 35°, considerable swelling can be obtained in the absence of ionophores or other permeability-modifying reagents (Fig. 2A). A comparison of the pH dependency of swelling in Na+ nitrate in the absence and the presence of gramicidin (to enhance electrophoretic Na+ permeability) shows that nitrate permeability also increases with increasing pH (Fig. 3; see Ref 7 also). It is possible that increased swelling at high pH in the absence of gramicidin is the result of cation movement in response to increased interior negative potential due to enhanced nitrate diffusion, rather than a pH-dependent alteration in cation permeability *per se*. However, isotopic turnover studies (9,10) indicate that monovalent cation mobility increases with

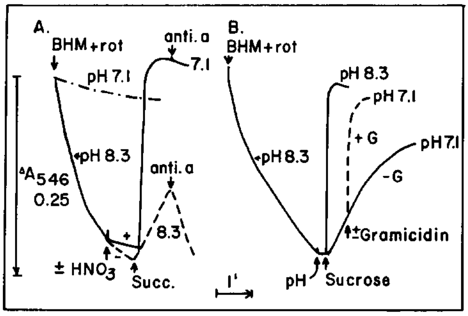


Fig 2 - Passive swelling of beef heart mitochondria in Na+ nitrate showing respiration-dependent contraction (A) and sucrose-dependent osmotic contraction (B). Beef heart mitochondria were prepared using a Polytron blender in 0.25M sucrose containing 0.5mM Tris EGTA, pH 7.1. Rotenone-treated mitochondria were suspended at 0.5 mg/ml in a medium of $NaNO_3$ (100mM), Tris (2mM, pH 7.1 or 8.3), EGTA (30μM), and sucrose (5mM) at 35° and the absorbance at 546 mμ recorded using an Eppendorf photometer. In A the contraction on addition of succinate (2mM) at pH 8.3 is compared with the reaction in which the pH was lowered to 7.3 with nitric acid. The effect of antimycin a (0.2 ug/mg) at the two pH values is also shown. In B the osmotic contraction following addition of sucrose (100mM) at pH 8.3 and 7.1 is shown. Where indicated gramicidin (1×10^{-7}M) was added.

increasing pH. Regardless of the exact mechanism, it is clear that considerable electrophoretic penetration of Na+ occurs under the conditions just specified. The penetration of Na+ is prevented by returning the pH to 7.0-7.2, so that the increased permeability seems completely reversible (Fig. 2A). Swelling in K+ nitrate requires a slightly higher pH to attain the same rate as in Na+ nitrate and an even more alkaline medium is necessary for the Li+ salt. Passive swelling in Mg^{+2} nitrate or in sucrose did not develop under pH 9. Mg^{+2} and other divalent cations (Ca^{+2}, Mn^{+2}) inhibited the swelling in $NaNO_3$ (cf Fig. 3) to a greater extent than other solutes at equal osmotic strength.

Mitochondria swollen under these conditions contract in an energy-dependent reaction upon addition of substrate (Fig. 2A) or exogenous ATP. The ability to swell mitochondria by simple pH manipulation without added reagents which modify the permeability of the membrane to cations, permits an analysis of the effects of cation permeability on the energy-dependent contraction reaction. In qualitative agreement with the results of Azzi and Azzone (4) who studied the

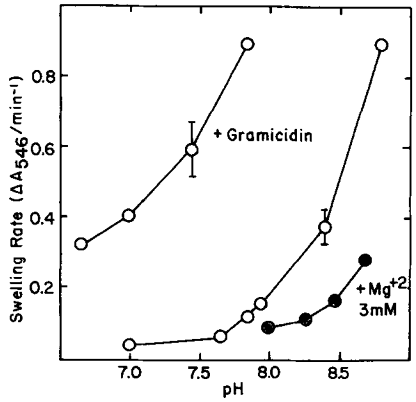


Fig 3 - Effect of pH on initial swelling rate of heart mitochondria suspended in sodium nitrate (100mM). The experimental conditions were identical to those of Fig 2 with pH adjusted by NaOH addition. Where indicated gramicidin (1×10^{-7}M) or Mg^{+2} nitrate (3mM) was also added.

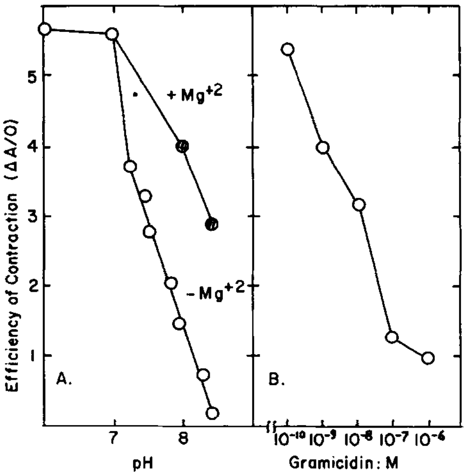


Fig 4 - The efficiency of energy-dependent contraction of mitochondria swollen as shown in Fig 2. The rate of contraction (ΔA) was estimated for the first 15-30 sec following addition of succinate (2mM) as was the rate of respiration (µatoms O_2/min./mg protein). The efficiency of respiration-dependent contraction is plotted as the quotient of these two values ($\Delta A/O$). Mg^{+2} was present at 3mM where indicated.

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contraction of valinomycin or EDTA-treated liver mitochondria, we have found that Na+ supports the contraction reaction better than K+, that contraction falls off with increased pH, and that contraction is favored by Mg^{+2} and inhibited by gramicidin. Since rates of respiration or ATP-utilization vary under these different conditions, we have analyzed the contraction reaction in terms of its efficiency (i.e. the absorbance change per O_2 or ATP consumed during the initial phase of the contraction). Efficiency of contraction in Na+ nitrate is maximal at about pH7.0 and falls off markedly as the pH is increased (Fig. 4A). In the presence of Mg^{+2}, which inhibits transmembrane electrophoretic permeability (Fig. 3), the decline in respiration-dependent contraction efficiency with increasing pH is much less pronounced (Fig. 4A). On the other hand, if increasing amounts of gramicidin are added at pH 7.2 to increase trans-membrane Na+ permeability, the efficiency of the contraction reaction decreases considerably (Fig. 4B).

Addition of sucrose (100 mM) to mitochondria swollen in $NaNO_3$ at pH 8.2 produces a rapid osmotic contraction at high pH where permeability to both anion and cation permits rapid outflow of accumulated salts in response to the osmotic pressure of the non-permeant sucrose (Fig. 2B). However, if the pH is adjusted to 7.1 before addition of sucrose, osmotic contraction is inhibited. This inhibition of osmotic contraction is enhanced by Mg^{+2} and released by gramicidin (Fig. 2B), results which strongly suggest that at neutral pH, low rates of cation efflux limit passive osmotic contraction in $NaNO_3$-loaded mitochondria.

DISCUSSION

The present studies clearly support an osmotic contraction model as opposed to a contractile mechanism to explain the energy-dependent extrusion of ions and water which decreased the volume of swollen mitochondria. The osmotic contraction model (Fig. 1B) postulates that the metabolism-dependent pH gradient can be utilized by a cation for H+ exchanger to bring H+ into the matrix and extrude Na+. Since H+ is neutralized by interior alkalinity, the exchange results in a decrease in net interior positive charge and an electrophoretic extusion of the permeant nitrate anion. Since Na+ (and nitrate) are being "pumped" out by this mechanism, an osmotic contraction will result. Increased permeability to Na+ would obviously decrease the efficiency of the contraction, since extruded Na+ could leak back into the interior. Passive swelling studies

(Fig. 2A and 3) show that trans-membrane Na+ permeability is increased by elevated pH and it has previously been established that gramicidin enhances (11) and Mg^{+2} inhibits (4) trans-membrane Na+ permeability. The fact that the efficiency of the contraction reaction is enhanced by Mg^{+2} (Fig. 4A) and decreased by gramicidin (Fig. 4B) or elevated pH (Fig. 4A) clearly is in line with the predictions of the osmotic contraction model.

In contrast, models for energy-dependent contraction of swollen mitochondria which suggest that a contractile change in the membrane results in iso-osmotic salt and water extrusion (Fig. 1A) predict that contraction will be favored by increased Na+ permeability. An analogy would be the sucrose-dependent passive osmotic contraction shown in Fig. 2B. In this reaction, addition of the non-permeant sucrose results in an osmotic imbalance and flow of water out of the swollen mitochondria. This water flow reverses the concentration gradients and, if Na+ and NO_3- can readily diffuse out of the matrix, further contraction occurs as a result of this osmotic contribution. In this case, increased permeability (gramicidin or elevated pH) results in more rapid contraction and decreased permeability (i.e. addition of Mg^{+2}) retards the reaction (Fig. 2B).

All of these considerations extend and confirm earlier indications obtained by Azzi and Azzone (4) using other swelling systems and clearly favor the osmotic contraction model (Fig. 1B). The more recent estimates of pore size published by Massari and Azzone (6) seem to be at odds with the present analysis and may require re-evaluation. In addition, a number of properties of the mitochondrial Na+/H+ exchanger (12) strongly suggest its involvement in the osmotic contraction process (Fig. 1B). These include a pH optimum of 7.2-7.3, little effect of Mg^{+2} on the exchange process (12,13), and a marked preference for Na+ over K+ in the exchange. A more complete account of these studies will be presented elsewhere.

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REFERENCES

1. Lehninger, A. L. (1962) Physiol. Rev. 42, 467-517.
2. Brierley, G. P. (1975) Molecular & Cellular Biochem., in press.
3. Brierley, G. P. (1974) Ann. N. Y. Acad. Sci. 227, 398-411.

4. Azzi, A. and Azzone, G. F. (1967) Biochim. Biophys. Acta 135, 444-453.
5. Nicholls, D. G. (1974) Eur. J. Biochem. 49, 585-593.
6. Massari, S. and Azzone, G. F. (1972) Biochim. Biophys. Acta 283, 23-29.
7. Brierley, G. P., Jurkowitz, M., Scott, K. M., and Merola, A. J. (1970) J. Biol. Chem. 245, 5404-5411.
8. Van Dam, K., Nieuwenhuis, F. J. R. M., and Steins, J. H. W. L. (1972) FEBS Sympos 28, 387-400.
9. Gamble, J. L., Jr. (1963) Proc. Soc. Exptl. Biol. 113, 375-377.
10. Diwan, J. J. (1973) Biochem. Biophys. Res. Communs. 50, 384-391.
11. Chappell, J. B. and Crofts, A. R. (1965) Biochem. J. 95, 393-402.
12. Wehrle, J. P., Jurkowitz, M., Scott, K. M., and Brierley, G. P. (1975) manuscript in preparation.
13. Wehrle, J. P. and Brierley, G. P. (1975) Federation Proc. 34, 542a.