SOME ASPECTS OF MITOCHONDRIAL STRUCTURE

J. POPINIGIS *, Y.TAKAHASHI **, T. WAKABAYASHI **, R. M. HULL and C. H. WILLIAMS
Institute for Enzyme Research and Department of Anesthesiology, University of Wisconsin, Madison, Wisconsin 53706, USA

Received 8 October 1971

1. Introduction

It has been suggested that the ultrastructural changes observed in mitochondria after the addition of protamine could be explained as the consequence of generating a higher ΔpH between the inner and outer mitochondrial compartments [1, 2]. This paper demonstrates that a similar direction of change in mitochondrial ultrastructure can be induced by lowering the pH of the medium. Moreover, the energized-twisted configuration (ET) of beef heart mitochondria, which was suggested to be an intermediate in the structural changes occurring during oxidative phosphorylation [3, 4], can also be induced under nonenergized conditions (rotenone + antimycin + oligomycin + arsenite) in pH 6.5 medium containing phosphate, butylmalonate and malate.

2. Materials and methods

Heavy beef heart mitochondria were prepared and suspended in 0.25 M sucrose + 10 mM Tris chloride pH 7.5 [5]. Mitochondria from pork liver were prepared and suspended in 0.25 M sucrose + 3 mM Tris chloride pH 7.3 [6]. Protein content was estimated by the biuret method [7]. Respiration was measured with a Beckman oxygraph equipped with a Clark oxygen electrode in 5 ml of reaction medium, pH 7.3, containing 15 mM KCl, 5 mM $MgCl_2$, 50 mM Tris chloride, 5 mM potassium phosphate, 3 μg of rotenone and 0.2 ml of mitochondrial suspension (heavy beef heart mitochondria – 10 mg of protein in exp. A or pork liver mitochondria – 12 mg of protein in exp. B and C.) After the addition of 5 mM Tris succinate respiration was recorded for 2 min. Oxygen consumption was 55 nanogram atoms O_2/min/mg of mitochondrial protein in exp. A and 25 nanogram atoms O_2/min/mg of mitochondrial protein in exp. B and C. Respiration was then completely inhibited by the addition of a solution of protamine sulfate at a concentration of 50 μg/mg of mitochondrial protein (exp. A and B) or by addition of HCl to pH 5.8 (exp. C). After 1 min of inhibitor action samples were fixed by mixing an equal volume of 2% glutaraldehyde in 0.25 M sucrose + 50 mM cacodylate buffer, pH 7.3 (exp. A M and B) or 2% glutaraldehyde in 0.25 M sucrose (exp. C) in the reaction chamber.

Exp. D was performed as described by Papa et al. [8]. Mitochondria from beef heart (5 mg of protein) were incubated in 1 ml of the pH 6.5 medium containing 200 mM sucrose, 20 mM Tris chloride, 1 mM $MgCl_2$, 0.5 mM EDTA, 3 μg of rotenone, 1.5 μg of antimycin, 10 μg oligomycin and 1 mM arsenite. After 2 min of preincubation of mitochondria 5 mM potassium phosphate was added followed 2 min later by addition of 5 mM butylmalonate with the subsequent addition of 5 mM Tris malate. At 6 min the experiments were terminated and fixed by mixing euqal vol. of 2% glutaraldehyde in 0.25 M sucrose + 50 mM cacodylate buffer pH 6.5. Procedures used for electronmicroscopy were described by Wakabayashi et al. [9]. Sectional specimens were examined in a Hitachi Hu 11B electronmicroscope operated at 75 KV.

* Permanent address: Department of Biochemistry, Medical School, Gdansk, Poland.
** Permanent address: Department of Pathology, Nagoya University School of Medicine, Nagoya, Japan.

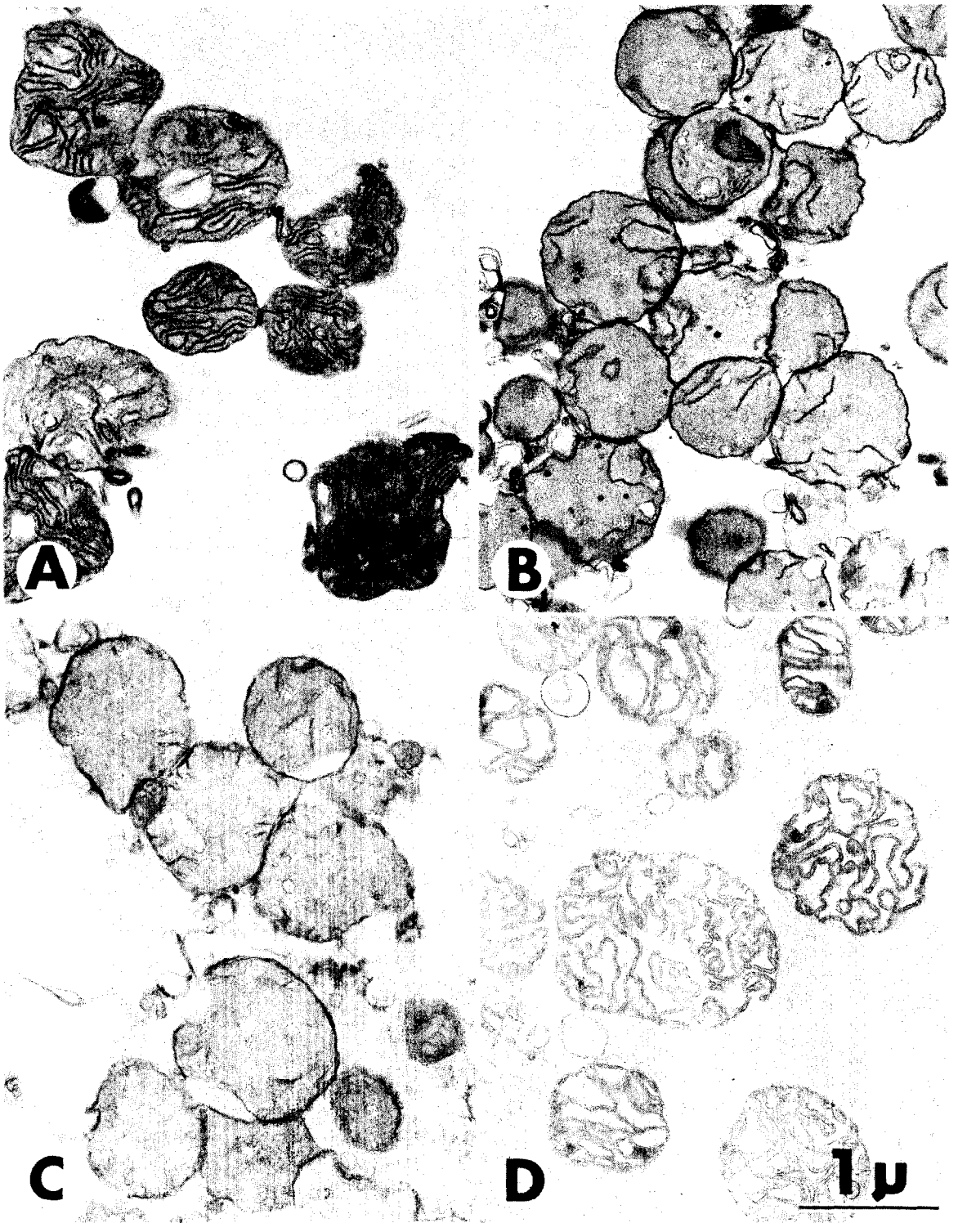

Fig. 1. Ultrastructure of mitochondria: A: Beef heart – succinate oxidation inhibited by protamine. B: Pig liver – succinate oxidation inhibited by protamine. C: Pig liver – succinate oxidation inhibited by lowering of pH of the medium to 5.8. D: Configuration of heart mitochondria named "energized twisted" (ET) obtained in pH 6.5 medium containing antimycin, rotenone, oligomycin, arsenite, by addition of phosphate, butylmalonate and malate.

3. Results and discussion

Protamine has been shown to be a potent inhibitor of mitochondrial respiration and it was suggested that this inhibition is a consequence of an increase of ΔpH between the inner and outer mitochondrial phase [10]. Recently this suggestion was supported by observations that the effect of citrate and uncouplers on respiration is similar in the case of protamine inhibition in intact mitochondria and "inside out" sonic particles (in the absence of protamine) in alkaline (pH 8.7) medium [11, 12]. Experimental evidence suggesting that protamine acts at the membrane level [1, 2, 10] seems to be in agreement with the observed specific action of this protein in developing action potential in black lipid films [13].

Protamine inhibition of respiration occurs with concomitant changes in mitochondrial structure [1, 2]. It is interesting that by using protamine it was possible to show structural similarities between heart mitochondria (fig. 1A) and liver mitochondria (fig. 1B), which normally show many structural differences. In both cases (fig. 1A and B) mitochondrial respiration (state 4 succinate + rotenone) was inhibited by addition of protamine and samples were fixed for electronmicroscopy. Typical changes as reported before – contraction of intermembrane space and cristae with matrix space expansion can be observed. A tendency for membrane opposition and matrix space expansion could be also observed under conditions when respiration was inhibited by lowering the pH of the medium to pH 5.8, although to a lesser degree (fig. 1C). Further increases in H^+ concentration in the medium causes the disruption of mitochondrial structure (not shown). The structure of these disrupted mitochondria seems to be similar to those observed as in the case of protamine + gramicidin [1,2].

The fact that mitochondrial ultrastructure is not simply related to the ΔpH is shown in exp. D. In this case isolated heart mitochondria were incubated in pH 6.5 medium containing antimycin, rotenone, oligomycin and arsenite. Next, by the addition of phosphate, butylmalonate and malate to these non-energized mitochondria, it was possible to induce ultrastructural transitions similar to those reported as a consequence of energization [3, 4]. At the present time it is difficult to suggest a mechanism for these structural changes, even though the effect of butylmalonate and changes in anion composition which can occur under those conditions are established [14–18, 8]. The mechanistic aspects are further complicated by the observation of anion accumulation in boiled mitochondria [19] and uncoupler and membrane transport inhibitor sensitive anion accumulation in matrix protein isolated from mitochondria [20, 21].

On the other hand, although the existence of "ET" configuration as one of the structural forms normally observed in mitochondria was questioned [22], it is our contention that data obtained from experiments *in situ* [23] and experiments performed with isolated mitochondria in the presence of membrane anion transport inhibitors [24] demonstrate that the "ET" configuration is not an artifact of fixation but is a consequence of biochemical reaction(s) which is (are) related to the anion and proton concentrations inside the mitochondrion.

Acknowledgements

These studies were supported in part by Program Grant No. GM-12847 from the National Institute of General Medical Sciences (USPHS) and Wisconsin Heart Association Grant 133-7571. We appreciate the expert technical assistance of Mrs. Alice Blado, the photographic work of Mr. Don Silver and the electron micrographs taken by Miss Ghousia Ghatala and Miss Nancy Lee.

References

[1] J. Popinigis, J. Swierczynski and T. Wrzolkowa, FEBS Letters 9 (1970) 309.
[2] J. Popinigis, T. Wrzolkowa and J. Swierczynski, Biochim. Biophys. Acta 245 (1971) 70.
[3] D. E. Green, J. Asai, R. A. Harris and J. T. Penniston, Arch. Biochem. Biophys. 125 (1968) 684.
[4] J. T. Penniston, in: Abstract V of VIIth International Congress of Biochemistry I-53, Tokyo, 1967.
[5] Y. Hatefi and R. L. Lester, Biochim. Biophys. Acta 27 (1958) 83.
[6] E. C. Weinbach, Anal. Biochem. 2 (1961) 335.
[7] A. G. Gornall, C. J. Bardawill and M. M. David, J. Biol. Chem. 177 (1949) 751.
[8] S. Papa, A. Francavilla, G. Paradies and B. Meduri, FEBS Letters 12 (1971) 285

[9] T. Wakabayashi, O. Hatase, D. W. Allmann, J. M. Smoly and D. E. Green, J. Bioenergetics 1 (1970) 527.
[10] J. Popinigis, W.Rzeczycki and J. Swierczynski, FEBS Letters 8 (1970) 149.
[11] J. Popinigis, R. F. Brucker, J. M. Smoly and C. H. Williams, submitted to FEBS Letters.
[12] J. M. Smoly and J. Popinigis, submitted to FEBS Letters.
[13] P. Mueller and D. O. Rudin, Nature 213 (1967) 603.
[14] F. Palmieri and E. Quagliariello, European J. Biochem 8 (1969) 473.
[15] S. Papa, N. E. Lofrumento, E. Quagliariello, A. J. Meijer and J. M. Tager, J. Bioenergetics 1 (1970) 287.
[16] B. H. Robinson and J. B. Chappell, Biochem. Biophys. Res. Commun. 28 (1967) 249.
[17] A. J. Meijer, in: Anion Translocation in Mitochondria (Academic Service, Amsterdam, 1971) p. 77.
[18] A. J. Meijer, in: Anion Translocation in Mitochondria (Academic Service, Amsterdam, 1971) p. 77.
[19] R. N. Zahlten, A. A. Hochberg, F. W. Stratman and H. A. Lardy, in preparation.
[20] J. M. Smoly, R. A. Capaldi and R. M. Hull, Federation Proc. 30 (1971) 1192 Abs.
[21] J. M. Smoly, in preparation.
[22] G. R. Hunter and G. P. Brierley, J. Cell. Biol. 50 (1971) 250.
[23] C. H. Williams, W. J. Vail, R. A. Harris, M. Caldwell, D. E. Green and E. Valdivia, J. Bioenergetics 1 (1970) 147.
[24] J. Popinigis, Y. Takahashi, T. Wrzolkowa and C. H. Williams, in preparation.