BBA Report

BBA 41199

'Crystal-like structures' of boiled beef heart mitochondria

J. POPINIGIS*, TERESA WRZOŁKOWA, Y. TAKAHASHI** and C.H. WILLIAMS

Department of Biochemistry and Laboratory of Electron Microscopy Medical School, Gdańsk (Poland) with Institute for Enzyme Research and Department of Anesthesiology, University of Wisconsin, Madison, Wisc. (U.S.A.)

(Received January 6th, 1972)

SUMMARY

Electron micrographs of boiled beef heart mitochondria are presented. On heating the mitochondrial suspension for 7 min in a boiling water bath 'crystal-like' structures inside and inbetween the mitochondria became visible.

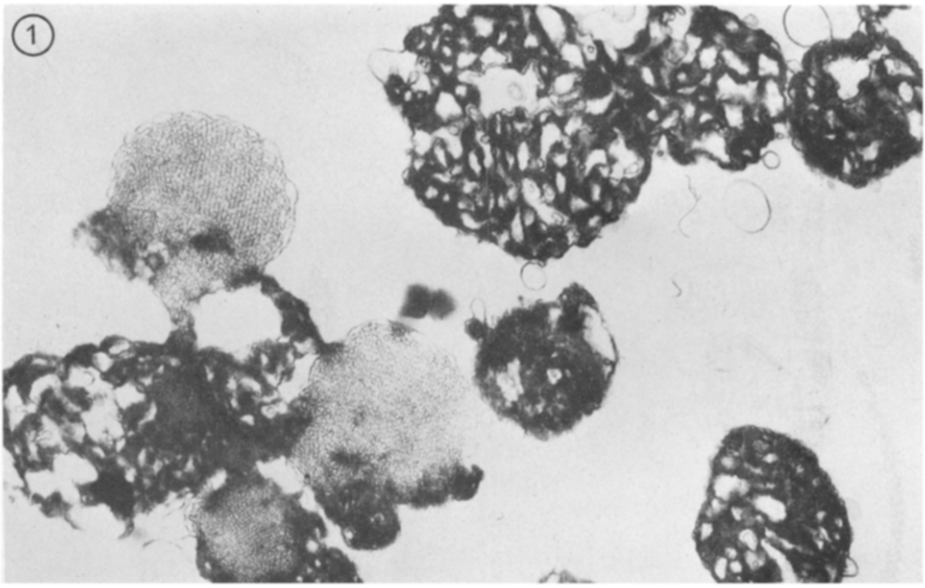
It is suggested that these structures were formed by rearrangement of the mitochondrial structure.

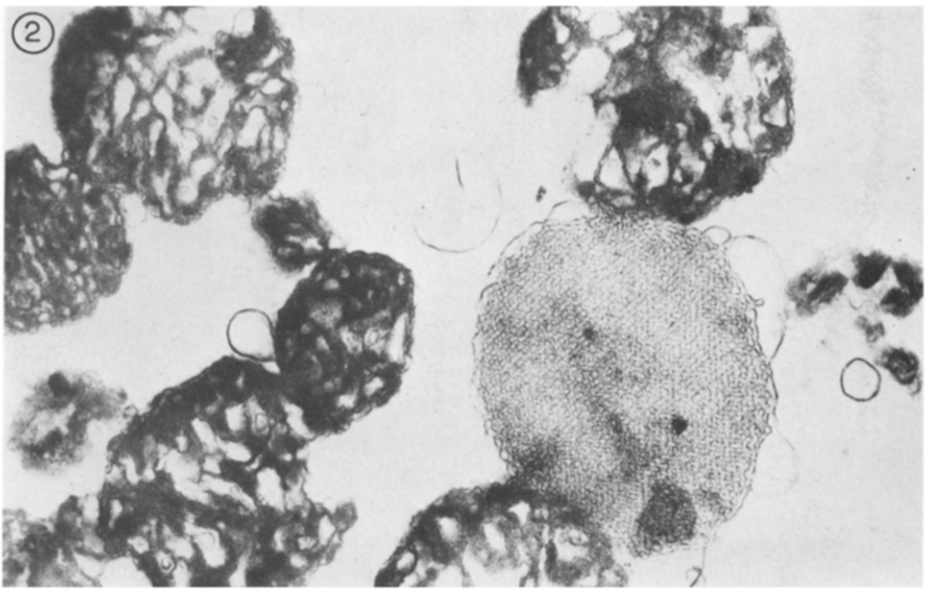
Recently we performed experiments showing that similar changes in mitochondrial ultrastructure, as observed upon energization, can be induced in beef heart mitochondria under nonenergized conditions (rotenone + antimycin + oligomycin + arsenite) in a medium of pH 6.5 by the addition of phosphate *plus* butylmalonate *plus* malate[1].

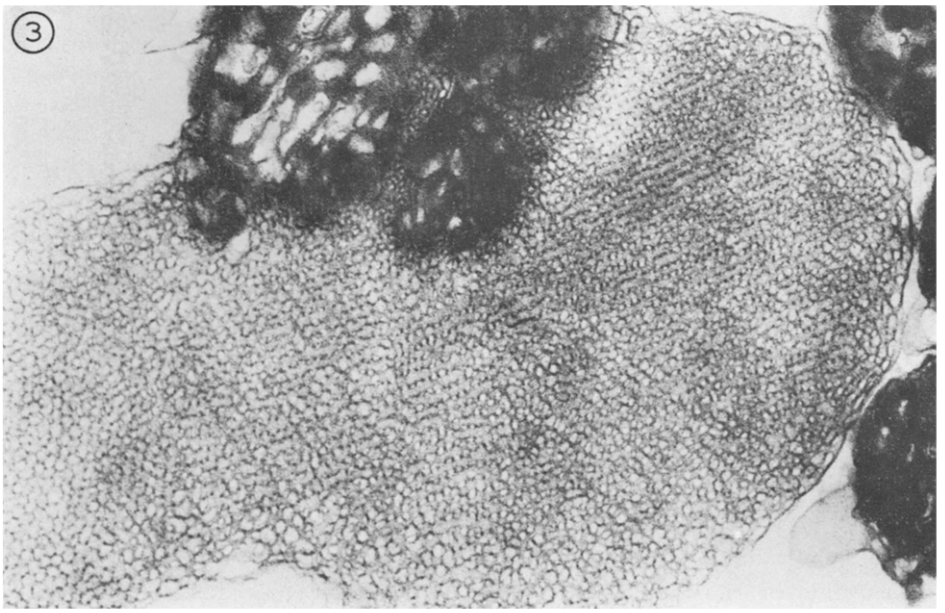
At the same time we were carrying out control experiments, using beef heart mitochondria boiled for 7 min at 100 °C. This type of experiment was necessary, as it has been observed that boiled mitochondria retain their ability to bind anions (R.N. Zahlten, A.A. Hochberg, F.W. Stratman and H.A. Lardy, unpublished). Although we could not observe distinct ultrastructural transitions in these mitochondria upon addition of anions, when analyzing the samples we observed the occurrence of structures not detectable under the same conditions in unboiled mitochondria. These 'crystal-like structures' are described in this communication.

*During the academic year 1970/1971 postdoctoral fellow, Institute for Enzyme Research, Madison, Wisc., U.S.A.

**Permanent address: Department of Pathology, Nagoya University, School of Medicine, Nagoya, Japan


1


2



Figs 1–3. 'Crystal-like' structures of boiled beef heart mitochondria. For experimental conditions see text. Magnifications: Fig. 1: 32 400 ×. Fig. 2: 32 400 ×. Fig. 3: 52 200 ×.

Heavy beef heart mitochondria were prepared[2] in 0.25 M sucrose + 10 mM Tris chloride, pH 7.5. Isolated mitochondria were suspended in the same solution with a final protein concentration of 50 mg/ml. Protein content was determined by the biuret method[3] The mitochondrial suspension (5 ml) was boiled in a water bath at 100 °C for 7 min and cooled in an ice bath. 0.2 ml of mitochondrial suspension was transferred to 1 ml of the pH 6.5 medium containing 200 mM sucrose, 20 mM Tris chloride, 5 mM potassium phosphate, 1 mM sodium arsenite, 1 mM $MgCl_2$, 0.5 mM EDTA, 3 μg rotenone, 1.5 μg antimycin and 10 μg oligomycin. The samples were incubated at 30 °C for 8 min and fixed by mixing with an equal volume of 2% glutaraldehyde in 0.25 M sucrose + 50 mM cacodylate buffer (pH 6.5) at 0°C. Procedures used for electron microscopy were as described by Wakabayashi *et al.*[4]. Specimens were examined in a JEM 7 A electron microscope at 80 kV.

The boiled mitochondria differ from intact mitochondria in an increased volume of the inner compartment, the electron density of the matrix, the structure of all mitochondrial membranes, and the presence of 'crystal-like' structures in and outside the mitochondria (Figs 1, 2, 3). It seems that in boiled mitochondria more membranous structures forming cristae are present than in intact mitochondria. Under higher magnification small multiple splittings of the cristal membrane are visible. Usually in these new formed 'splitting spaces' a substance of low electron density is present. In some places of the inner compartment splitting membranes form a small network of crystal-like

structures. Most of the 'crystal-like structures' appear close to the mitochondria, seldom separated. They are formed by many threads, situated more closely to each other in the central part of structure. The outer part of these 'crystal-like' forms is less compact and often we can see vesicular structure incorporated into the network (Fig. 2). Sometimes the outer part of the 'crystal-like structure' is limited by the membrane-like structure (Fig. 2). Every single thread in the described 'crystal-like' structure has many, regularly distributed small empty spaces, limited on both sides by small knob-like foci of higher electron density (Fig. 3). In the 'crystal-like structures' between threads electron dense substances are present.

It is our contention that the described 'crystal-like' structures derive from the mitochondria. This possibility is supported by the following observations:

(1) Inside some of the mitochondria there are small foci of 'crystal-like' structures of different size and different stages of development.

(2) Most of the 'crystal-like' structures show a connection with mitochondria.

(3) Our 'crystal-like' structures are similar to the 'lattice structures' observed in the intracristal space of intact beef heart mitochondria upon addition of phosphotungstic acid [5,6].

(4) 'Paracrystalline array patterns' inside the mitochondria were reported by other authors[7-9].

(5) A crystalline prolamellar body in the plastid[10] shows some similarities to 'crystal-like' structure.

The significance of 'crystal-like' structures can only be speculated upon[11], but the possibility that these structures are involved in ultrastructural transitions of mitochondria may be considered. According to Hackenbrock[12] energization leads to the rearrangements of matrix protein from the tightly packed configuration in the low-energy state to an expanded lattice in the high-energy state.

REFERENCES

1 J. Popinigis, Y. Takahashi, T. Wakabayashi, R.M. Hull and C.H. Williams, *FEBS Lett.*, 19 (1971) 221
2 Y. Hatefi and R.L. Lester, *Biochim. Biophys. Acta*, 27 (1958) 83.
3 A.G. Gornall, C.J. Bardawill and M.M. David, *J. Biol. Chem.*, 177 (1949) 751.
4 T. Wakabayashi, O. Hatase, D.W. Allman, J.M. Smoly and D.E. Green, *J. Bioenerg.*, 1 (1970) 527.
5 T. Wakabayashi, *Fed. Proc.*, 30 (1971) 1017 Abstr.
6 T. Wakabayashi and J.M. Smoly, in the press.
7 K. Blinzinger, N.B. Newcastle and H. Hager, *J. Cell Biol.*, 25 (1965) 293.
8 E.H. Newcomb, M.W. Steer, P.K. Hepler and W.P. Wergin, *J. Cell Biol.*, 39 (1968) 35.
9 C.H. Williams, W.J. Vail, R.A. Harris, M. Caldwell and D.E. Green with E. Valdivia, *J. Bioenerg.*, 1 (1970) 147.
10 D. von Wettstein, K.W. Henningsen, J.E. Boynton, G.C. Kannangara and O.F. Nielsen, in N.K. Boardman, A.W. Linnane and R.M. Smile, *Autonomy and Biogenesis of Mitochondria and Chloroplasts*, North-Holland Publ. Co., Amsterdam, London, 1971, p. 205.
11 E.F. Korman, R.A. Harris, C.H. Williams, T. Wakabayashi and D.E. Green, *J. Bioenerg.*, 1 (1970) 387.
12 C.R. Hackenbrock, *Proc. Natl. Acad. Sci. U.S.*, 61 (1968) 598.