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Paracrystalline Arrays in Mitochondria following Ageing of Mitochondria *in situ*

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Abstract

Paracrystalline arrays of helical configuration were observed in the mitochondrial intracrystal spaces following prolonged ageing of mitochondria *in situ*. The occurrence of these mitochondria with the paracrystalline arrays, average diameter of about 70 Å, appeared to increase following an increase in the time of ageing *in situ*.

The exact function of the mitochondria containing the paracrystalline arrays is unknown. These mitochondria could not possibly be responsible for the overall decline in the State 3 respiration, respiratory control index and the ADP/O ratio observed with intact mitochondria isolated after prolonged ageing *in situ* [21].

Introduction

Various forms of mitochondrial inclusions [1-9] have been reported in different types of tissue [1, 10-12] occurring in both normal [6, 11, 17] and in pathological [7-8, 13-16] conditions. These inclusions could occur as crystals [11], paracrystalline array patterns either in the form of "Star of David" [1, 10] or of long "helical structure" [1, 17-18], lamellar arrays [12] and dense lines [6]. The mitochondrial inclusions could be localized either in the matrix [10, 12, 19] or in the intracristal space [1, 11, 17] of the mitochondria.

This paper reports the formation of paracrystalline arrays of helical configuration in the mitochondrial intracristal spaces following prolonged ageing of mitochondria *in situ*. The inclusions were randomly distributed within the mitochondria, with some mitochondria containing

more than one inclusion. The average width of the helical paracrystalline array was about 70 Å, with wide variations in length.

Methods

Ox-neck muscle, after prolonged storage at 4°C, was cut into approximately 0.5 mm cubical blocks and then fixed in 2.5% glutaraldehyde in 200 mM phosphate buffer (pH 7.1) containing 200 mM sucrose before being stained with 1% osmium tetroxide. The small blocks of skeletal muscle, embedded in Epon 812 [20], were cut into thin sections on an LKB Ultratome III using glass knives and then stained with uranyl acetate and lead citrate before examination with an electron microscope.

Mitochondria from porcine back-muscle, both fresh and stored at various time intervals post-mortem [21] at 1°C, were isolated using Nagarse *Bacillus subtilis* [22]. The fixation and treatment of the mitochondrial samples for thin sections were carried out as described by Allmann *et al.* [23] but without using acrolein, and Epon 812 was used for embedding. The sections were examined using both the AEI (Model EM6-B) and the Philips (Model EM 300) electron microscopes at 60 or 80 Kv.

Results and Discussion

Mitochondria isolated intact from either the ox-neck or porcine back-muscle after prolonged ageing *in situ* showed a decline in the State 3 respiration, respiratory control index and ADP/O ratio [21-22]. The percentage decline in these parameters depended on the rate of decline in tissue pH [21-22]. Mitochondria capable of carrying out oxidative phosphorylation could still be isolated from aged tissue as long as the tissue pH was maintained at or above 5.5 [21]. Electron microscopic examinations of various preparations of mitochondria previously aged *in situ* revealed the presence of paracrystalline arrays in the intracristal space. The occurrence of these structural inclusions appeared to increase as the time of mitochondrial ageing was increased. These paracrystalline inclusions were randomly distributed within the mitochondria, with some mitochondria containing more than one of these structures.

Figure 1 shows the appearance of three paracrystalline arrays running parallel to each other within a mitochondrion isolated from the porcine (Large White) back-muscle after 48 hours ageing *in situ*. These structures consisted of a central core, appearing as a row of spherical particles, and were surrounded on either side by an electron-transparent zone, which in

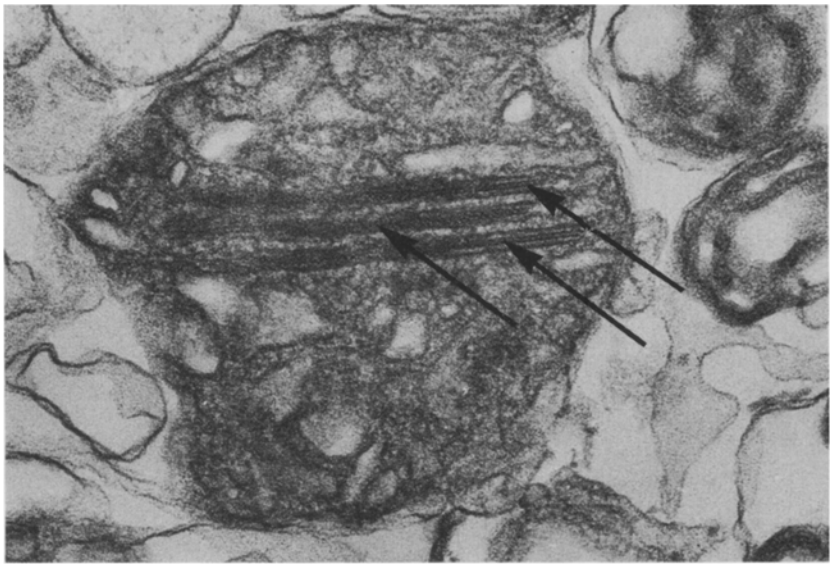

Figure 1. Electron micrograph illustrating three paracrystalline arrays in a single mitochondrion. The mitochondrion was isolated from the porcine (Large White) back-muscle after 48 h ageing *in situ* at 1° C in a polythene bag. Final magnification: 80,000 ×.

turn was bounded by an electron dense membrane frequently observed to be continuous with the mitochondrial inner membrane surrounding the mitochondrial intracristal space. The characteristics of the paracrystalline structure are illustrated more clearly in Fig. 2 where the mitochondrion had only a single inclusion. The electron micrograph also reveals that the paracrystalline array probably had a helical configuration, judging from the marked transition from a continuous elongated (A) to a discontinuous (B) structure.

The ultrastructure of the paracrystalline array was of a helical configuration. This was demonstrated using the Philips (Model EM 300) electron microscope fitted with a goniometer stage, thereby giving the facility of tilting the specimen through an angle of ± 60° with respect to the incident beam. Under these conditions the appearance of the paracrystalline array depended on the angle of tilt. Figure 3 illustrates the appearance of four randomly distributed paracrystalline arrays within a single mitochondrion when the specimen was tilted through an angle of +20° (A) to an angle of −30° (B). The clearly marked transition of a continuous (Fig. 3A, I) to a discontinuous (Fig. 3B, I) paracrystalline array, and the display of both of these structural arrangements of

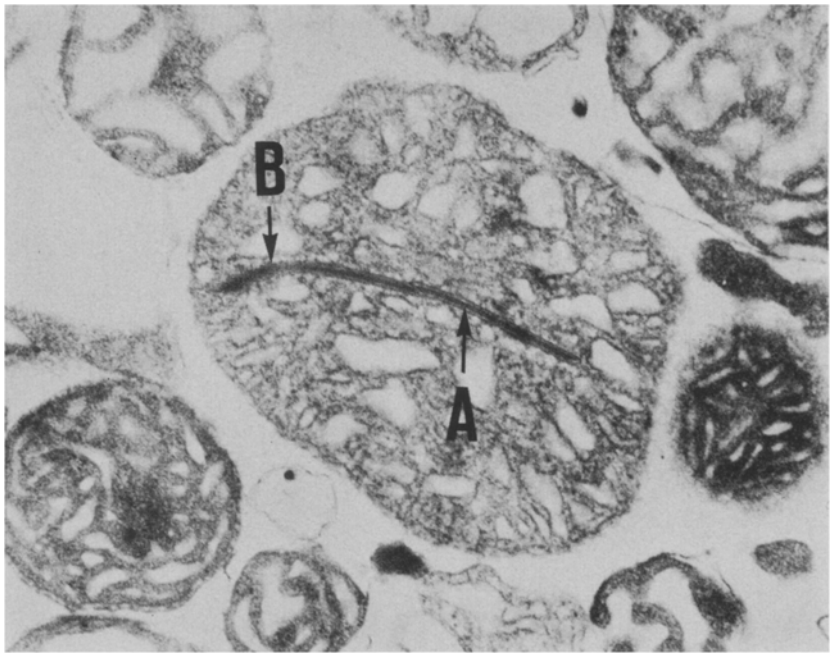

Figure 2. Electron micrograph showing a single paracrystalline array in a mitochondrion after 24 h ageing *in situ* at 1° C. Final magnification: 70,000 ×.

the paracrystalline arrays within the same mitochondrion (Fig. 3B, III and IV) could only be possible if the paracrystalline array were in a helical configuration. The loss in the resolution of the paracrystalline arrays III and IV (Fig. 3A) was due to both of these inclusions not being in the same plane with the other two paracrystalline arrays I and II in the mitochondrion. Microdensitometric measurements on the negatives of the electron micrographs from five samples gave an average width of the helical paracrystalline array of about 70 Å, with a separation space of about 110 Å on either side of the paracrystalline array.

Intracristal paracrystalline arrays were also observed in the mitochondria of ox-neck muscle after being subjected to a period of prolonged ageing. The occurrence of these mitochondrial inclusions *in situ* (Fig. 4) and in the isolated mitochondria (Fig. 1-3) from aged tissue indicates that these structural inclusions were most unlikely to be artefacts. The exact function of the mitochondria containing the paracrystalline arrays is unknown. These mitochondria could not possibly be responsible for the decline in the State 3 respiration, respiratory control index and the ADP/O ratio observed with intact mitochondria isolated after prolonged

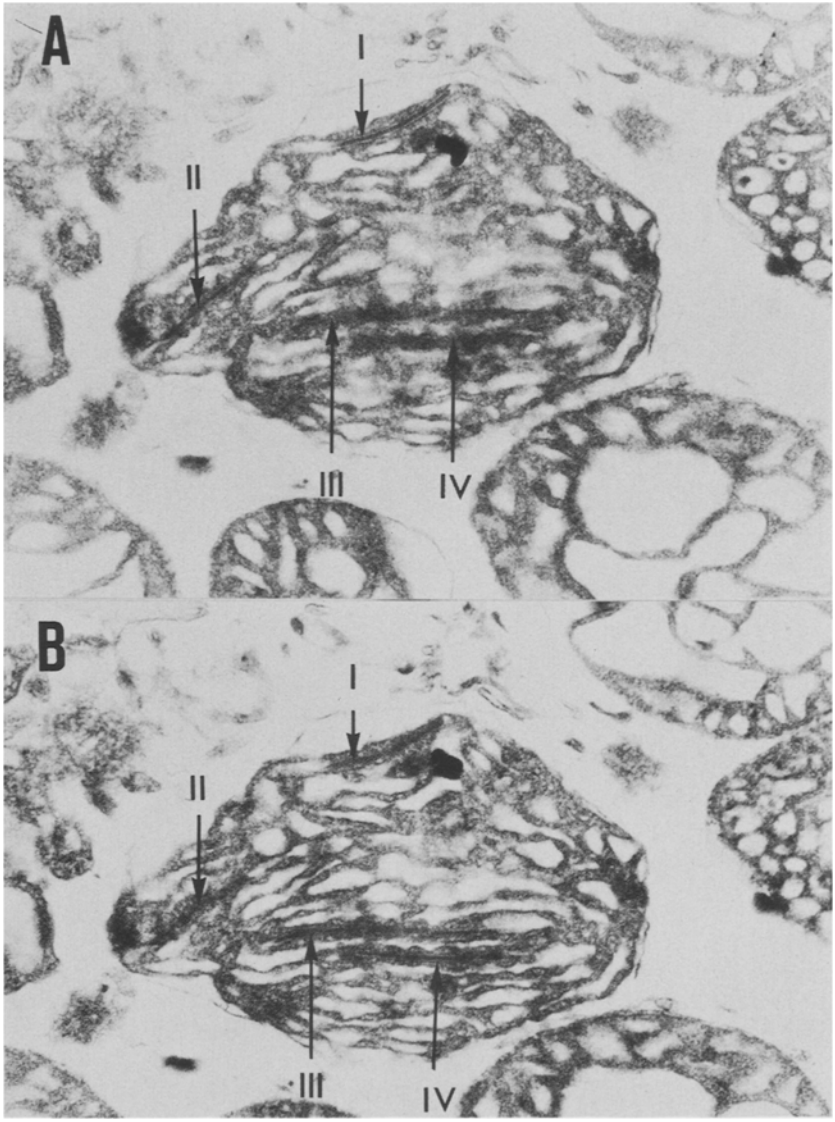

Figure 3. Electron micrographs illustrating the helical configuration of the paracrystalline arrays. The single mitochondrion, isolated from the porcine (Large White) back-muscle after 24 h ageing *in situ*, contained four paracrystalline arrays, designated I-IV. Electron micrographs were taken with a Philips (Model EM 300) electron microscope fitted with a goniometer stage. A, +20° tilt; B, −30° tilt. Final magnification: 70,000 ×.

Figure 4. Electron micrograph showing the paracrystalline arrays in mitochondria *in situ* after 120 h ageing at 4° C. Final magnification: 40,000 x.

ageing *in situ* [21], as the mitochondria containing the paracrystalline arrays contributed less than 10% of the mitochondrial population in a single electron microscopic field. These mitochondria with the helical paracrystalline arrays could be responsible for other biochemical properties not yet determined.

A similar paracrystalline array in the intracristal space of beef heart mitochondria was also recently observed [24]. This structure was referred to as the intracristal protein network which under appropriate conditions of compression of the crista formed helical arrays. The ageing effect of the isolated mitochondria described above could be due to the formation of the orthodox configuration, a configuration which favoured the lateral compression of the network system (see Ref. 24).

Several mechanisms for the formation of the mitochondrial inclusions had been postulated by various investigators (see Ref. 12). Some of the hypotheses include the suggestion of interference of the basic enzymatic activity within the mitochondria [9] and of changes in the phospholipid content [5, 25]. Formation of lamellar arrays in the mitochondrial matrix of pregnant rat liver could also be induced by the administration of pregnenolone-16-α-carbonitrile [12].

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