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Collapse and Extension of the Headpiece-Stalk Projections in Mitochondrial Electron Transport Particles

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Fernandez-Moran, Oda, Blair and Green [1] who in 1964 first demonstrated repeating structures in the mitochondrial inner membrane by negative straining emphasized that aging of mitochondrial specimens was essential for satisfactory visualization of 90 Å spherical headpieces separated from the membrane by 50 x 30 Å cylindrical stalks. More recently, the intensive study of configurational transitions in beef heart and adrenal cortex mitochondria has uncovered evidence that the headpiece-stalk projections are extended to variable extents in different configurational states [2-5]. Thus in the orthodox configuration of adrenal cortex mitochondria, the headpieces appear to be fully extended [2]; in the paired configuration of beef heart mitochondria (induced under energizing conditions), the headpieces appear to be collapsed atop the membrane [3, 4]; and in the fused configuration of beef heart mitochondria, the headpiece-stalks appear to be incorporated into the membrane [5]. Packer has also recently reported evidence from freeze-fracture studies that headpieces may exist within the membrane

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continuum [6]. The basis of the variability in respect to the extension and collapse of headpiece-stalks has been an enigma up to now. The present communication provides evidence that the headpiece-stalk projections of submitochondrial inner membrane preparations can exist in two conformations—collapsed and extended—and that these two optional conformational states can be controlled by appropriate ions and treatments.

Experimental

Preparation of submitochondrial particles. ETP_H was prepared from beef heart mitochondria by the method of Hansen and Smith [7] and used without storage in the critical experiments. We have found that ETP_H stored at $-20°$ for weeks or months can still duplicate the essential findings applicable to freshly prepared ETP_H.

Electron microscopy. All samples were fixed by addition of glutaraldehyde to a final concentration of 0.1%. The mixture was allowed to stand for 2 min at room temperature before negative staining with phosphotungstic acid according to the method of Brenner and Horne [9]. The stained samples were examined in a Hitachi HU-11B or HU-11E electron microscope operated at 75 KV.

Results

ETP which is a relatively uncoupled submitochondrial particle (P/O ratios of 0.1 or less) shows consistently a pattern in negative staining of arrays of *extended* headpiece-stalks (see Fig. 1). The headpieces are clearly separated from the membrane as evidenced by two criteria: (a) the spherical headpieces form sets which are parallel with the inner membrane and separated from the membrane by a gap of some 50 Å; (b) in favorable instances the cylindrical, 30 Å wide stalk can be seen connecting headpiece to membrane. Wherever headpiece-stalks are clearly visualizable as in ETP not only are the arrays observed edgewise but the surfaces of the vesicles are covered with *discrete* 90 Å particle.

ETP_H which can achieve close to theoretical coupling (P/O ratio approaching 2.0 with succinate as substrate) shows a significantly different pattern in negative staining (see Fig. 2). First there are relatively few vesicles with regular arrays of extended headpieces (frequency less than 5%). Second, the headpieces predominantly appear to merge into the membrane. Moreover, the surface of these vesicles show particles which are considerably larger than the 90 Å headpiece—a token that these are composed of sets of several headpieces. We shall refer to the pattern in ETP_H as the collapsed conformation and the pattern in ETP as the extended conformation of the headpieces.

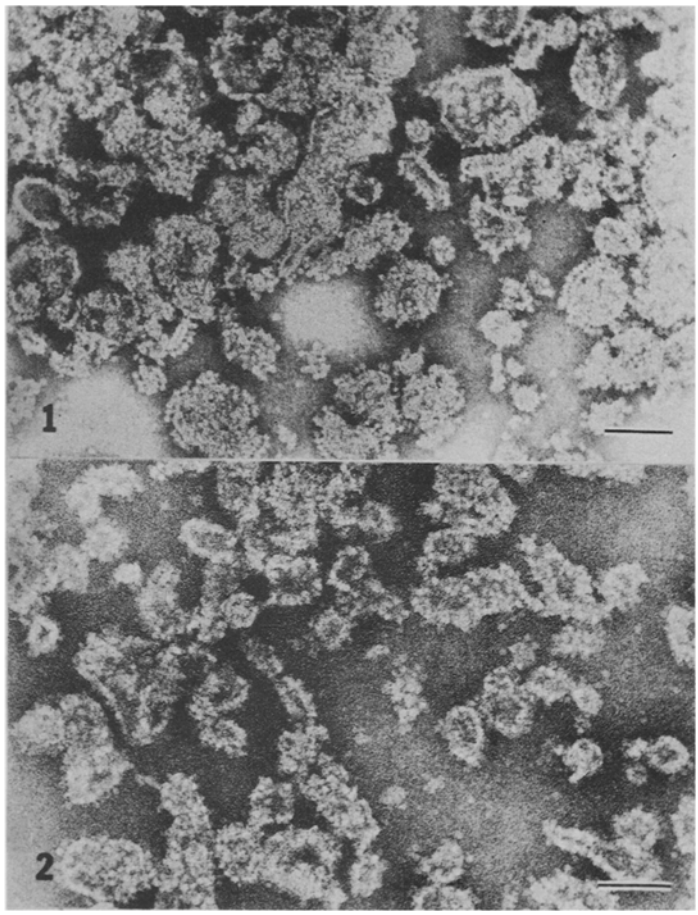

Figure 1. Negatively stained ETP.

Figure 2. Negatively stained ETP_H.

When ETP_H was allowed to age in a 0.25 M sucrose medium, 10 mM in Tris-Cl of pH 7.4, for 2 h at 30° it showed a pattern in negative staining indistinguishable from that of ETP (see Fig. 3), namely a profusion of vesicles with arrays of extended headpieces and connecting stalks.

The same transition in pattern could be achieved by exposing ETP_H to sonic irradiation (Benson Sonifier at maximum output) for 30 sec at 0° in a sucrose medium (0.25 M) containing 1 mM ethylene diamine tetraacetate and adjusted to pH 8. The sonicated suspension was centrifuged at 144,000 x g for 30 min and the sedimented particles were resuspended in fresh sucrose media. We shall refer to this modified

particle as resonicated ETP_H, abbreviated as RS-ETP_H. When negatively stained, RS-ETP_H showed a pattern indistinguishable from that of ETP or aged ETP_H (see Fig. 4). That is to say the headpieces were predominantly in the extended conformation. Coupling was not completely abolished as a result of sonication. The P/O ratio declined from initial values of 1.4 or higher to values of about 0.6. When RS-ETP_H was exposed to energizing conditions (supplementation of the sucrose medium with 10 nM ATP, 5 nM succinate, 5 nM Mg^{++} and 10 nM Mn^{++}) the pattern in negative staining was indistinguishable from that of untreated ETP_H (see Fig. 5). The key additions in energization

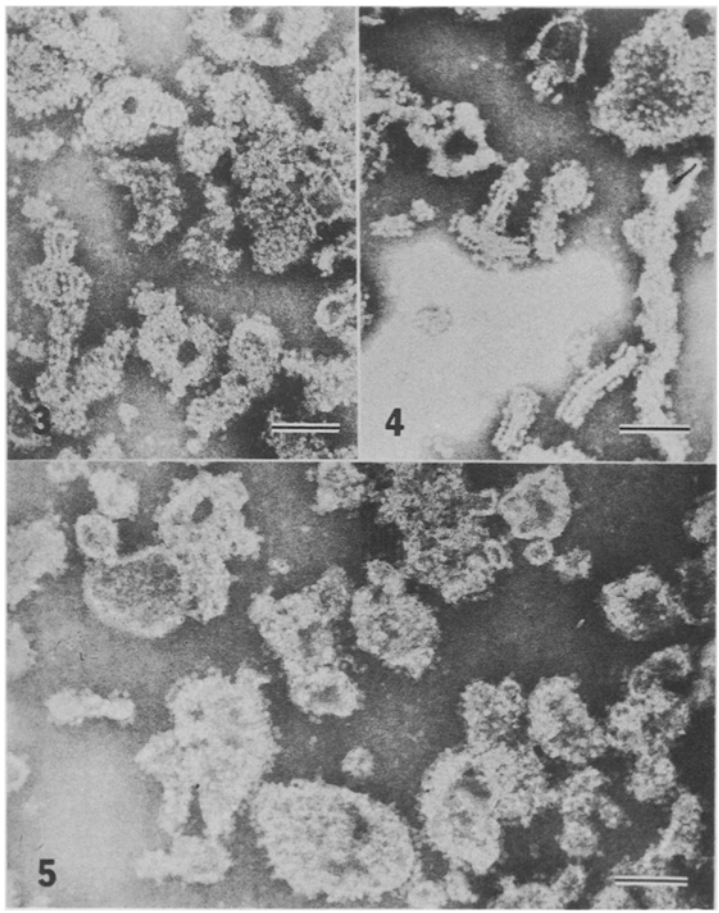

Figure 3. Negatively stained aged ETP_H. Figure 4. Negatively stained RS-ETP_H.
Figure 5. Negatively stained RS-ETP_H under energizing conditions.

were ATP (or ADP) and the divalent metals. Either ATP alone or the divalent metals alone was less effective than the combination of the two. ATP plus divalent metals was virtually equivalent to ATP plus succinate plus divalent metals.

This restoration of the collapsed conformation of the headpiece by energization of RS-ETP_H could not be achieved with ETP. Apparently some component required for this restoration by energization was missing in ETP.

Discussion

The electron microscopic studies reported above establish three points: (a) that there are indeed two optional conformations of the headpiece-stalk projections of the inner membrane, namely extended as in ETP and collapsed as in ETP_H; (b) that energization can induce a transition from the extended to the collapsed conformation of the headpiece-stalks in the appropriate particle (namely resonicated ETP_H); and that in certain particles (ETP and ETP_H) the conformation of the headpiece-stalk projections is frozen and unaffected by energizing conditions.

Since ETP is uncoupled with respect to oxidative phosphorylation [10] and ETP_H is coupled [11], it is probably very meaningful that the headpiece-stalk projections are extended in the uncoupled particle and collapsed in the coupled particle. Coupling of electron transfer to synthesis of ATP appears to require the tightest apposition of headpiece with membrane–an apposition which involves the disappearance of the stalk.

Mitochondria *in situ* undergo a transition from the orthodox to the paired configuration when energizing conditions are imposed [12]. Concomitant with this configurational transition, the headpiece-stalk projection oscillates from the extended to the collapsed conformation [2-4]. This means that there is a control mechanism in normal mitochondria which regulates the conformation of the headpiece-stalk projection in the transition from nonenergizing to energizing conditions. This control mechanism is operative in resonicated ETP_H but not in ETP or ETP_H. Although the conformation of the headpiece-stalk projection is different in ETP as compared to ETP_H, in neither of these particles does energization affect the conformation of the headpiece-stalk projection. Since RS-ETP_H–a particle derived from ETP_H by sonication–is competent in respect to the control of the conformation of headpiece-stalk projection by energization, it would appear that the capacity for control of conformation is latent in ETP_H. The presence of a "collapse" factor is, thus, a necessary but not sufficient condition for exercise of control. Whatever the nature of the factor and/or state required for control, it is present in mitochondria and resonicated ETP_H and lacking or latent in ETP and ETP_H.

The headpiece-stalk has recently been shown by Tzagoloff [13] to be a projection from a membrane-forming complex which is not one of the complexes of the electron transfer chain. How the stalk fits into or is attached to the basepiece is still unknown. The stalk could slide in and out of the basepiece during collapse and extension of the headpiece or alternatively the stalk could undergo appropriate shape changes which would achieve the same collapse and extension cycle.

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