ULTRASTRUCTURAL STUDIES OF BEEF HEART MITOCHONDRIA

II. ADENINE NUCLEOTIDE INDUCED MODIFICATIONS OF MITOCHONDRIAL MORPHOLOGY

By N. E. Weber and P. V. Blair
with the Technical Assistance of Barbara Martin
Department of Biochemistry, Indiana University Medical Center
Indianapolis, Indiana 46202

Received September 11, 1970

Summary. Low levels of ADP, dADP, ATP and dATP induce a distinctive ultrastructure in the network of inner mitochondrial membranes whilst oxygen consumption is essentially unchanged. Induction of a coalesced membrane morphology by these adenine nucleotides is not prevented by oligomycin or low temperature (0° C), but is blocked by atractyloside. Other purine and pyrimidine nucleotides will not substitute for adenine nucleotides. These observations strongly indicate that gross morphological transitions of the inner membranes induced by adenine nucleotides are not intermediate energy transducing forms of oxidative phosphorylation, but probably reflect the consequences of specific and stoichiometric binding of adenine nucleotides to the translocation sites of the inner mitochondrial membrane.

Ultrastructural changes in membrane morphology, induced by transitions in steady state metabolism, have been assigned specific transducing roles in the conservation of oxidative energy during formation of adenosine triphosphate (1-3). It has been reported recently (4,5) that adenosine diphosphate alone will promote coalescence of inner mitochondrial membranes whilst oxygen uptake is unchanged. Stoner and Sirak (6) and Weber and Blair (7) have shown that low levels of ADP will transform condensed cristae into coalesced, multi-faceted membrane networks. In agreement with this finding in isolated mitochondria Hackenbrock (8) has demonstrated that ADP generated *in vivo* will cause a morphological change in mitochondria of metabolizing tissue.

This communication defines the nucleotide specificity and adenine nucleotide concentration required to induce coalescence of the inner mitochondrial membranes. Furthermore, it is shown that atractyloside prevents the adenine nucleotide induced membrane transformation but oligomycin and low temperature (0° C) do not block coalescence of the cristal membranes.

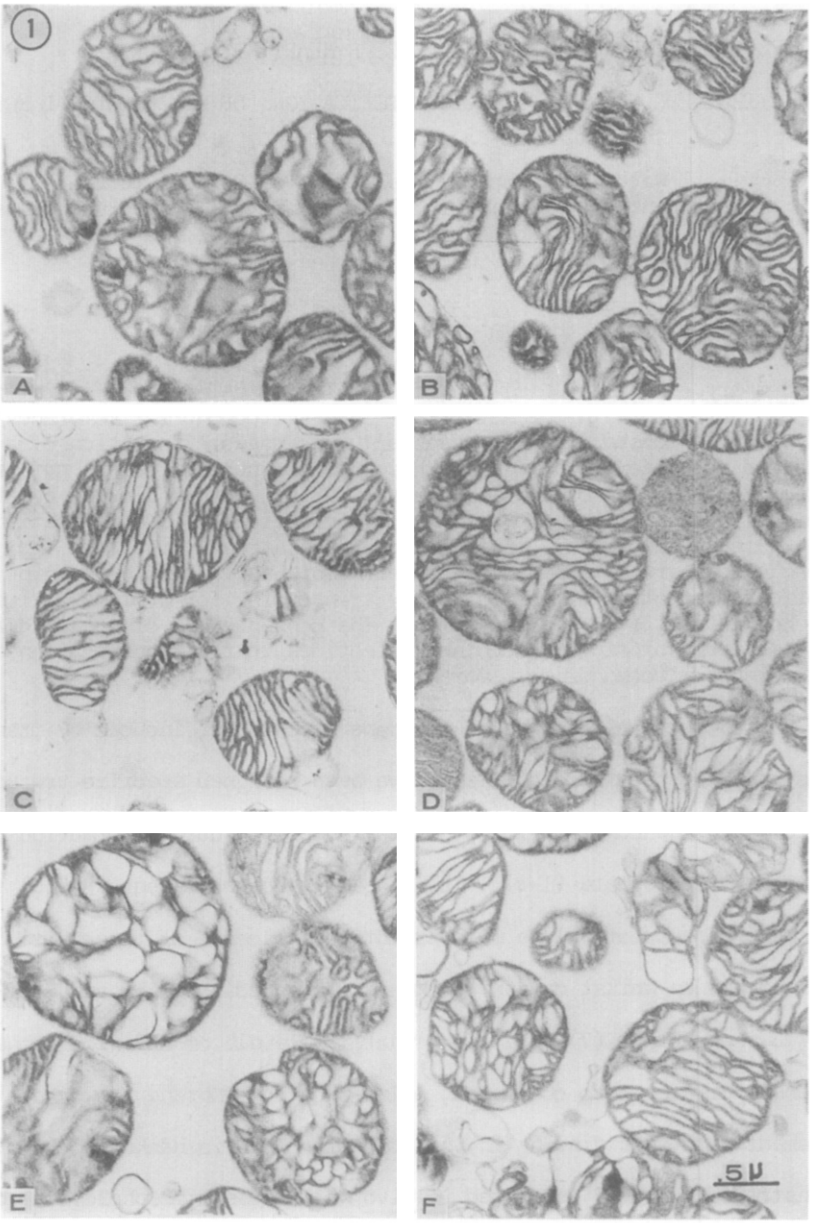

Fig. 1. Beef heart mitochondria were incubated with 5 mM pyruvate plus 0.5 mM malate in a solution containing 250 mM sucrose, 5 mM tris-HCl (pH 7.5) and 1 mg protein per ml. Two ml of each sample were removed after the following treatments: (A) Incubation at 30° for 1 min; (B) Incubation at 0° for 1 min; (C) Incubation at 30° for 1 min followed by addition of 4 nmoles of ADP, then incubation for 1 min; (D) Incubation at 30° for 1 min followed by addition of 4 nmoles of dADP, then incubation for 1 min; (E) Incubation at 30° for 1 min followed by addi-

tion of 6 nmoles of ATP, then incubation for 1 min; (F) Incubation at 30° for 1 min followed by addition of 4 nmoles of dATP, then incubation for 1 min. These aliquots were mixed immediately with 8 ml of a solution that was 0.25 M in sucrose, 2% in glutaraldehyde and 0.05 M cacodylate (pH 7.5). Samples remained in this fixative at room temperature for 15-30 min. The remaining steps in the electron microscopy procedures were those described by Penniston et al. (3). Specimens were examined with a modified RCA EMU-2 electron microscope.

Methods and Materials. A modification of the method of Crane, Glenn and Green (9) was used to isolate beef heart mitochondria. Further purification was accomplished by the procedure of Hatefi and Lester (10). Experimental details are described in legends to figures and tables. Protein concentrations were determined by the method of Gornall et al. (11). Electron microscopy was carried out by the methods described previously (4).

Results. Morphological "configurations" of the inner mitochondrial membrane have been described by Penniston et al. (3). The thickness of the inner mitochondrial membranes (double-layered cristae) in the "energized" (condensed) state is approximately 250 Å, whereas, the thickness of the cristae in the "non-energized" (coalesced and aggregated) state is approximately 125 Å. Mitochondria incubated at 0° and 30° in the presence of oxidizable substrate are illustrated in Fig. 1. The cristae are predominantly condensed or "energized".

Low levels of ADP, dADP, ATP and dATP transformed the mitochondrial membranes from the condensed to a coalesced, aggregated arrangement (Fig. 1C-1F) without appreciably changing the oxidation rates (Table I). ATP and dATP showed equal transforming capabilities and both diphosphates were equally effective at a low concentration (2.0 μM) of nucleotide. However, the diphosphates were considerably more efficient inducers of coalescence and aggregation than the triphosphates.

Mitochondria incubated with pyruvate plus malate and oligomycin (1 μg/mg protein) were transformed from a condensed to a coalesced, aggregated morphology by ADP (Fig. 2A and C). Atractyloside prevented this

TABLE I

Conditions: Oxidation rates were determined polarographically with a Gilson Medical Electronics Oxygraph. Pyruvate 15 mM and malate 1.5 mM were substrates in all reactions. One mg of mitochondrial protein was added to the 2 ml reaction mixture which was 250 mM in sucrose and 5 mM in Tris-HCl (pH 7.5). Reagents were included in the reaction mixture at the initial concentrations or levels indicated: 15 mM P_i, 0.25 mM ADP, 0.25 mM dADP, 50 µg atractyloside, and 1 µg oligomycin. Reactions were run at 30° C.

Additions	µatoms oxygen/min/mg protein
None	32.8
P_i	56.9
ADP	31.6
dADP	31.2
P_i + ADP (state III)	209.9
P_i + atractyloside + ADP	56.9
P_i + oligomycin + ADP	43.0

morphological transformation induced by ADP (Fig. 2E). When mitochondria were incubated with oxidizable substrate, inorganic phosphate (P_i) and either atractyloside or oligomycin the "energized-twisted" (distended cristae) configuration was generated (Fig. 2B). Oligomycin did not prevent reversion of the cristae to a condensed form when ADP was added, but conversion to a coalesced, aggregated morphology was not observed when P_i was present (Fig. 2D). Again, atractyloside prevented the ADP induced membrane transition i.e., mitochondrial cristae remained distended (Fig. 2F).

Electron micrographs (Fig. 3) illustrate that membrane transformations (condensed to coalesced) induced by adenine nucleotides also occur at 0°. Ultrastructural changes were not effectively induced by AMP, UDP, GDP, IDP, cyclic AMP, adenosine or adenine.

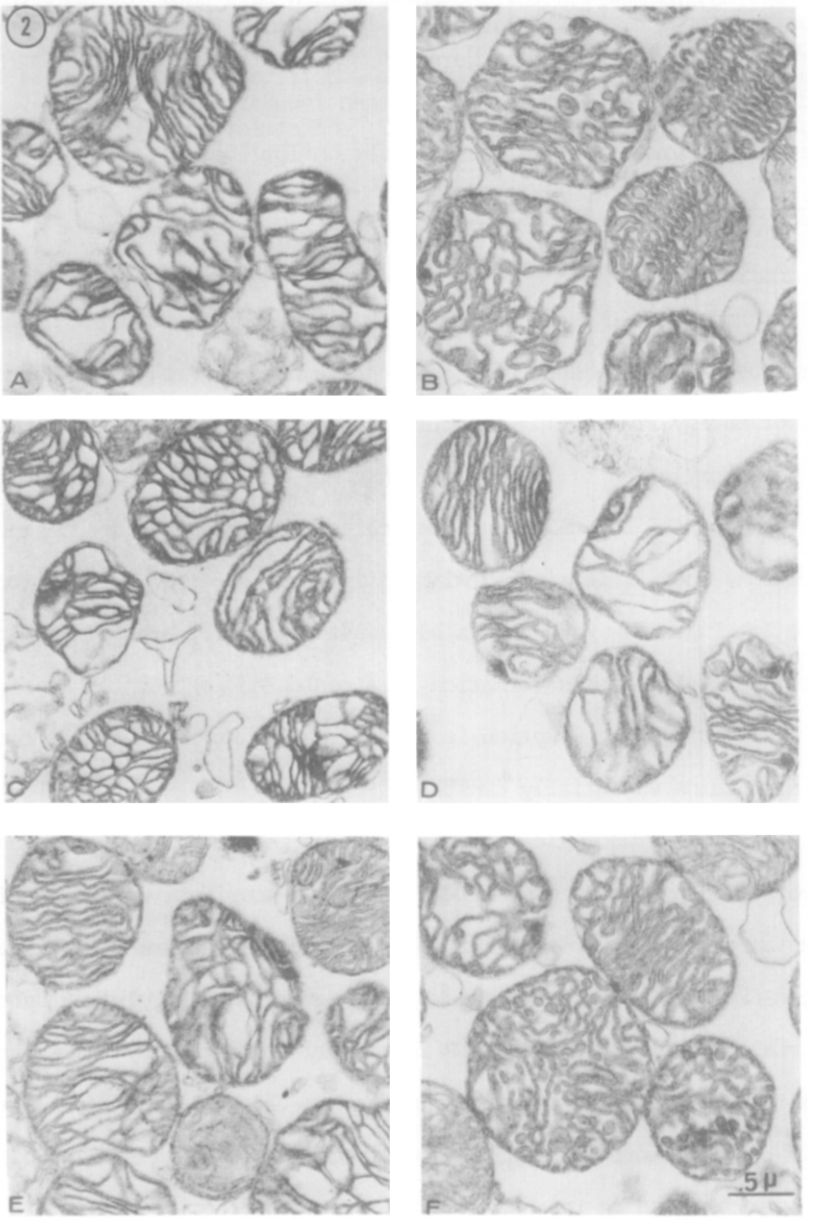

Fig. 2. Procedures, conditions and substrates were the same as those described for Fig. 1. Incubations were carried out at 30° and samples were removed after the following treatments: (A) Incubation for 2 min. with substrate and oligomycin (1 μg/mg protein)- mitochondria incubated with substrate and atractyloside (50 μg/mg protein) were indistinguishable from these; (B) Incubation for 2 min with substrate, P_i (15 mM) and atractyloside- mitochondria incubated with substrate, P_i and oligomycin were indistinguishable from these; (C) Incubation for 2 min with

substrate and oligomycin followed by addition of 1 μmole of ADP, then incubation for 1 min; (D) Incubation for 2 min with substrate, P_i and oligomycin followed by addition of 1 μmole of ADP, then incubation for 15 sec; (E) Incubation for 2 min with substrate and atractyloside followed by addition of 1 μmole of ADP, then incubation for 1 min; (F) Incubation for 2 min with substrate, P_i and atractyloside followed by addition of 1 μmole of ADP, then incubation for 15 sec.

Discussion. The present studies further support the postulate (4) that morphological changes in the inner mitochondrial membrane network, induced by oxidizable substrate and adenine nucleotides, are not expressions of energy transducing molecular conformations required for oxidative phosphorylation (1,3,12). Not only ADP but also dADP, and to a lesser extent ATP and dATP generate coalesced, aggregated membrane configurations when P_i is minimal without increasing oxygen consumption (Table I; Fig. 3). When oxidizable substrate and P_i are present the addition of dADP induces formation of a coalesced, aggregated configuration whilst oxygen consumption is only slightly increased and P_i concentration remains essentially constant.

The gross morphological transformations caused by the four adenine nucleotides are similar even though the deoxy- derivatives are non-functional in oxidative phosphorylation by intact mitochondria (13). However, dADP functions as a phosphate acceptor in phosphorylating submitochondrial particles (14) and dATP is rapidly hydrolyzed by these particles, and by the isolated adenosine triphosphatase (13,15). Therefore, it seems likely that the morphological changes induced by adenine nucleotides arise from specific binding (perhaps to the translocase) or transport of the nucleotide outside of the domain of coupled phosphorylation rather than function as energy transducing intermediates of oxidative phosphorylation.

The inhibition of ADP-induced membrane reorganizations by atractyloside and the failure of oligomycin to inhibit these changes when P_i is absent strengthens the idea that ADP driven morphological transitions are closely related to adenine nucleotide binding at the translocation site.

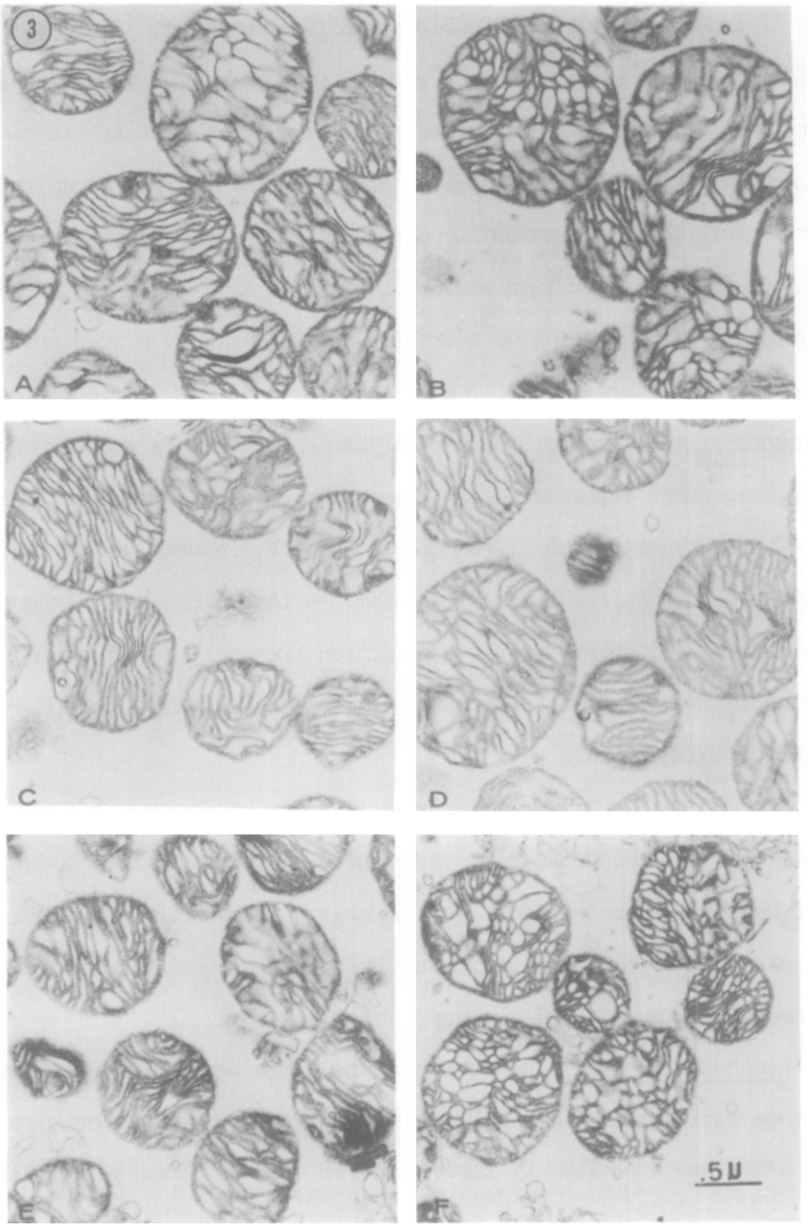

Fig. 3. Procedures, conditions and substrates were the same as those described for Fig. 1. Incubations were carried out at 0° and samples were removed after the following treatments: (A) Same as 1C except temperature was 0°; (B) Same as 1D except temperature was 0°; (C) Same as 1E except temperature was 0°; (D) Same as 1F except temperature was 0°; (E) Incubation for 1 min with substrate and P_i (15 mM); (F) Incubation for 1 min with substrate and P_i followed by addition of 1 μmole of ADP, then incubation for 1 min.

The observation that adenine nucleotides cause coalescence and aggregation of inner mitochondrial membrane at low temperature (0°), in the presence of oxidizable substrate and P_i, whilst oxidative phosphorylation is minimal or non-existent furnishes additional evidence in support of the notion that these morphological transformations arise from forces exerted outside of the oxidative phosphorylation apparatus. These transitions in membrane structure are not inconsistent with adenine nucleotide translocation and binding by heart (16) and liver (17) mitochondria. On the other hand, formation of the P_i-induced distended configuration does not occur at low temperature which probably indicates a requirement for energy-dependent transport of P_i across the cristal membranes. The failure of atractyloside and oligomycin to block P_i-induced distention of cristae which requires oxidizable substrate is consonant with the postulate that P_i transport, driven by electron transfer, should be unaffected by a nucleotide transport inhibitor (atractyloside) and a phosphorylation inhibitor (oligomycin).

In conclusion, we have presented a number of observations which indicate that mitochondrial inner membrane transitions in morphology, induced by adenine nucleotides, are not energy transducing (capturing) intermediates of oxidative phosphorylation but are coincident with and dependent upon nucleotide binding or translocation.

Acknowledgements. The authors acknowledge use of the electron microscope facilities of Dr. Ralph Jersild of the Department of Anatomy. This study was supported by three U.S. PHS Grants (HE06308, AM13939-01 and T01-GM-00360-08) and an Indiana Heart Association Grant.

References.

1. Hackenbrock, C. R., J. Cell. Biol., 30, 269 (1966).
2. Hackenbrock, C. R., Proc. Nat'l. Acad. Sci. U.S., 61, 598 (1968).
3. Penniston, J. T., Harris, R. A., Asai, J. and Green, D. E., Proc. Nat'l. Acad. Sci. U.S., 59, 624 (1968).
4. Weber, N. E. and Blair, P. V., Biochem. Biophys. Res. Comm., 36, 987 (1969).
5. Weber, N. E. and Blair, P. V., Ind. Acad. Sci., 79, (Abstract), 1969.

6. Stoner, C. D. and Sirak, H. D., Fed. Proc., 29, 734 (1970).
7. Weber, N. E. and Blair, P. V., Second CRM-ACS, (Abstract), 1970.
8. Hackenbrock, C. R. and Rehn, T., Fed. Proc., 29, 734 (1970).
9. Crane, F. L., Glenn, J. L. and Green, D. E., Biochim. Biophys. Acta., 22, 475 (1956).
10. Hatefi, Y. and Lester, R. L., Biochim. Biophys. Acta., 25, 83 (1958).
11. Gornall, A. G., Bardawill, C. J. and David, M. M., J. Biol. Chem., 177, 751 (1949).
12. Harris, R. A., Penniston, J. T., Asai, J. and Green, D. E., Proc. Nat'l. Acad. Sci. U.S., 59, 830 (1968).
13. Kemp, A. (Jr.) and Groot, G. S. P., Biochim. Biophys. Acta., 143, 628 (1967).
14. Low, H., Vallin, I. and Alm, B., In B. Chance, Energy-linked Functions of Mitochondria, Academic Press, New York, 1963.
15. Pullman, M. E., Penefsky, H. S. and Racker, E., Arch. Biochem. Biophys., 78, 227 (1958).
16. Brierley, G. P. and O'Brien, R. L., J. Biol. Chem., 240, 4532 (1965).
17. Pfaff, E. and Klingenberg, M., European J. Biochem., 6, 66 (1968).