OXIDATIVE PHOSPHORYLATION AND RESPIRATORY CONTROL IN LYSOLECITHIN TREATED ELECTRON TRANSPORT PARTICLES

Douglas R. Hunter, Hirochika Komai and Robert A. Haworth

Institute for Enzyme Research
University of Wisconsin
Madison, Wisconsin 53706

Received December 17, 1973

SUMMARY: Lysolecithin treatment of electron transport particles (ETP) generated non-vesicular fragments of membrane that can catalyze oxidative phosphorylation. Electron micrographs of ultrathin sections of lysolecithin treated ETP were devoid of circular patterns characteristic of closed vesicular structures. No synergistic uncoupling of oxidative phosphorylation by valinomycin plus nigericin in the presence of K^+ was observed in such fragments of membrane, which remained sensitive to classical uncouplers and to oligomycin. Preceding total destruction of closed vesicular structure, lysolecithin caused a drastic alteration in the membrane as evidenced by a greatly diminished effect of the ionophores in releasing respiratory control.

In a previous communication from this laboratory, we have shown that non-vesicular fragments of membrane generated by lysolecithin treatment of electron transport particles (ETP) can catalyze uncoupler sensitive ATP-$^{32}P_i$ exchange reaction (1). The small fragments of membrane obtained after a brief centrifugation of lysolecithin treated ETP had negligible NADH oxidase activity and phosphorylation coupled to NADH oxidation could not be measured.

Prior to centrifugation, however, we found that lysolecithin treated ETP retained low but measurable NADH oxidase activity. In the present communication we wish to report the results of experiments on oxidative phosphorylation and respiratory control in uncentrifuged, lysolecithin treated ETP.

MATERIALS AND METHODS

ETP were prepared as described earlier (1). Oxygen uptake was measured at 30°C using a Beckman oxygen analyzer. For the determination of respiratory control, the reaction mixture (4 ml) contained sucrose, 0.25 M; Tris-HCl (pH 7.4), 10 mM; KCl, 10 mM; particles (0.5 mg/mg) and NADH, 1 mM to start the reaction. Lysolecithin, 30 mg/ml in water, was added to the reaction flask

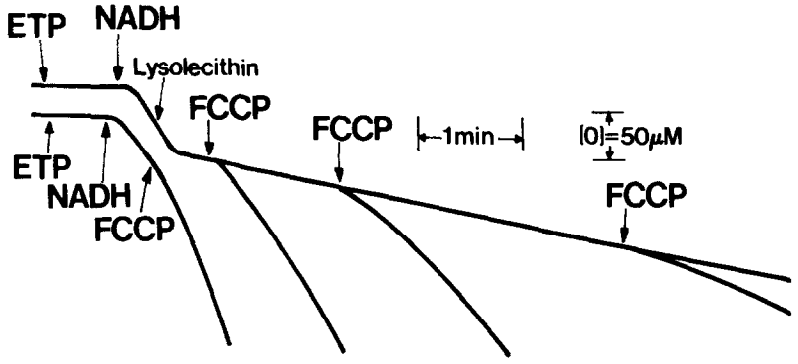

Figure 1. Time course of the inactivation of NADH oxidase activity by lysolecithin. The figure is a composite of traces obtained from several experiments in which the time intervals between addition of lysolecithin (1 mg/mg protein) and that of FCCP (3×10^{-7} M) were varied.

after 0.5 minutes. Then, after one additional minute, unless otherwise noted, alcoholic solutions of uncouplers or ionophores were added. The oxidative phosphorylation assay mixture (5 ml) contained: sucrose, 0.25 M; Tris-HCl (pH 7.4), 10 mM; $MgCl_2$, 3 mM; ADP, 1 mM; potassium phosphate containing $^{32}P_i$ (pH 7.4), 4 mM (ca. 4×10^5 CPM/μmole); glucose, 5 mM; hexokinase, 20 units/mg; NADH, 2 mM; and particles, 0.4 mg/ml and other additions as indicated. Phosphate esterification coupled to substrate oxidation was measured by withdrawing aliquots from the reaction mixture at intervals and determining the esterified ^{32}P after removal of inorganic phosphate (2). Radioactivity was measured as described earlier (1). Protein was determined by the method of Lowry *et al.* (3). Electron micrographs of ultrathin sections were obtained according to the method described earlier (1). Lysolecithin prepared from egg lecithin (Sigma) was used. Nigericin and carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) were a generous gift from Dr. Henry A. Lardy. All other chemicals used were obtained from commercial sources.

RESULTS AND DISCUSSION

The NADH oxidase activity of submitochondrial particles is strongly inhibited by lysolecithin (4). Ten seconds after addition of lysolecithin to the reaction mixture, the rate of NADH oxidation dropped to 30% of the rate in

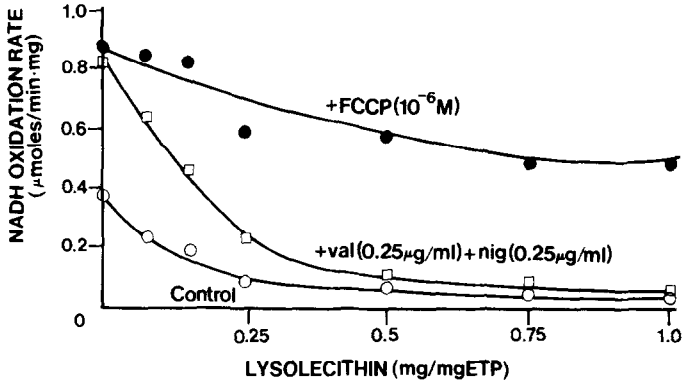

Figure 2. Effect of lysolecithin on the sensitivity of NADH oxidase activity of ETP to FCCP and to ionophores. Experimental procedure was described in Materials and Methods.

the absence of lysolecithin, and decreased very slowly from this level (see Figure 1). On the other hand, the rate of NADH oxidation in the presence of an uncoupler, FCCP, decreased much more slowly, and the result was an increased "respiratory control index." The sensitivity of ETP to FCCP did not decrease upon addition of lysolecithin. The concentration of FCCP required for 50% stimulation of respiration of both untreated ETP and lysolecithin treated ETP was about 10^{-7} M. In sharp contrast, the sensitivity of ETP to the ionophores, valinomycin and nigericin, was greatly decreased by lysolecithin. Concentrations of ionophores that released respiratory control as effectively as FCCP in untreated ETP rapidly became ineffective in releasing respiratory control upon addition of lysolecithin (see Figure 2).

For the measurement of oxidative phosphorylation, we treated ETP (10 mg/ml) in a medium 0.25 M in sucrose and 10 mM in Tris-HCl (pH 7.8) with lysolecithin (1.2 mg/mg protein)at 0°C for 30 minutes to insure complete destruction of closed vesicular structure. The NADH oxidase activity of such lysolecithin treated ETP was about 0.06 μmole/min.mg, or approximately 9% of the activity of untreated ETP. Nevertheless, the efficiency of oxidative phosphorylation was only slightly less than that of untreated ETP. As can be seen in Table I, oxidative phosphorylation catalyzed by lysolecithin treated ETP was sensitive to uncouplers

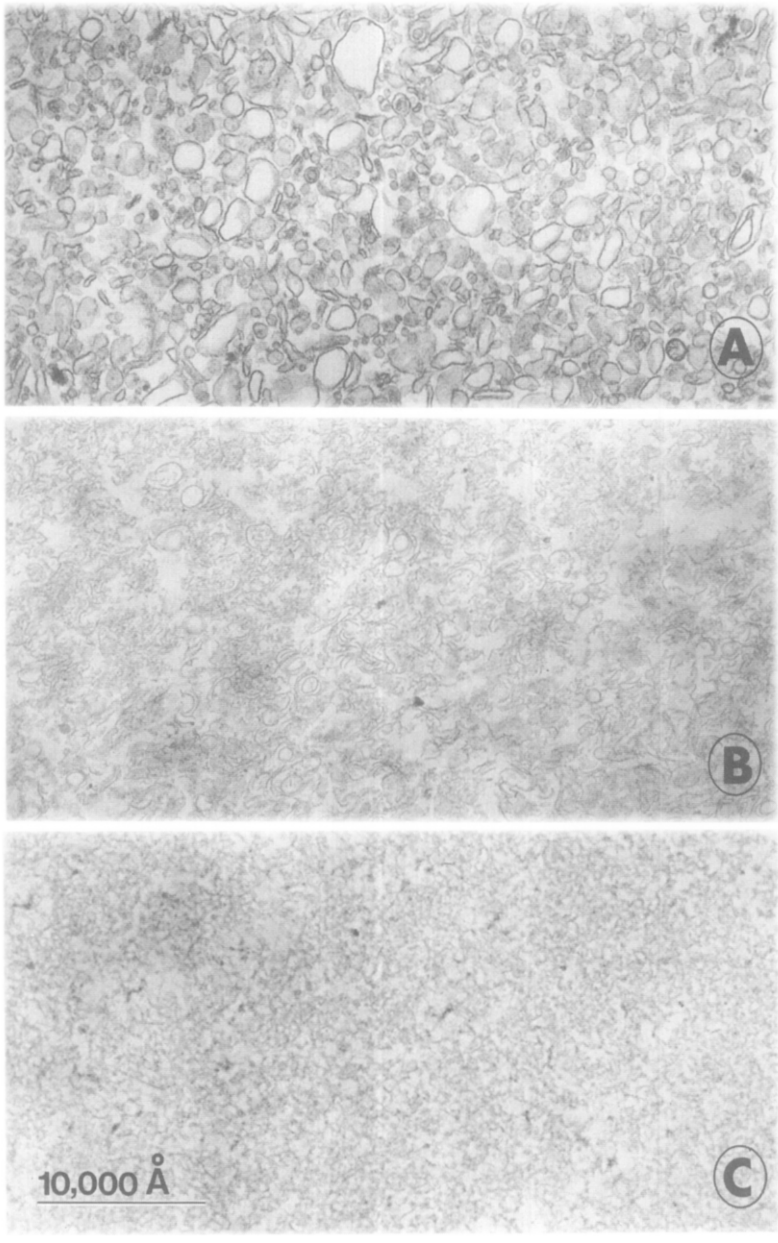

Figure 3. Electron micrographs of positively stained, ultrathin sections of (3a) ETP, (3b) lysolecithin treated ETP (1 minute, 30°C at 1 mg lysolecithin/mg protein) and (3c) lysolecithin treated ETP (30 minutes, 0°C at 1.2 mg lysolecithin/mg protein).

and to oligomycin. Note that the rate of NADH oxidation catalyzed by ETP treated with lysolecithin for 30 minutes was still enhanced by uncouplers. On the other hand, there was no synergistic inhibition by valinomycin plus nigericin in the

TABLE I. Effect of Uncouplers and Oligomycin on Oxidative Phosphorylation Catalyzed by Lysolecithin-Treated ETP.

Addition	Initial rate of NADH oxidation (units/mg)	P/O
None	0.06	0.49
DNP (1.6×10^{-4} M)	0.17	0.05
FCCP (2.4×10^{-7} M)	0.18	0.07
S_{13} (4.0×10^{-7} M)	0.24	0.01
Oligomycin (0.8 µg/ml)	0.05	0.00

Abbreviations: DNP = 2,4-dinitrophenol; FCCP = carbonylcyanide p-trifluoromethoxyphenylhydrazone; S_{13} = 5-chloro-3-*tert*butyl-2′-chloro-4′-nitrosalicylanilide.

presence of K^+ of the lysolecithin treated ETP (see Table II). (The inhibition by nigericin alone was almost the same as the inhibition with both nigericin and valinomycin together.) Incorporation of $^{32}P_i$ into organic phosphorus was negligible in the absence of an oxidizable substrate, indicating that the amount of hexokinase used was sufficient to eliminate $^{32}P_i$ incorporation due to adenylate kinase reaction and ATP-$^{32}P_i$ exchange reaction. The results contained in Table I and Table II were obtained with NADH as the substrate. Similar results were obtained with durohydroquinone as substrate.

At least two phases of change in the structure and functions of ETP were caused by lysolecithin. The first, rapid phase, which takes place within about 10 seconds after addition of lysolecithin to ETP, was characterized by inhibition of NADH oxidation in the absence of an uncoupler and greatly diminished effect of the ionophores in releasing respiratory control. Electron micrographs of ultrathin sections after 1 minute of treatment of ETP with lysolecithin showed major disruption of vesicular structure (Figures 3a and 3b). The second phase was considerably slower and involved total disruption of closed vesicular

TABLE II. Effect of Ionophores on Oxidative Phosphorylation.

Particles	Addition	P/O
ETP	None	0.59
	Val	0.53
	Nig	0.45
	Val + Nig	0.13
Lysolecithin-treated ETP	None	0.49
	Val	0.42
	Nig	0.30
	Val + Nig	0.28

Val = Valinomycin (1 μg/ml); Nig = Nigericin (0.2 μg/ml).

structure as indicated in Figure 3c. After 30 minutes of treatment of ETP with lysolecithin, electron micrographs showed no circular patterns and oxidative phosphorylation measurements showed complete absence of synergistic inhibition by the ionophores in the presence of K^+.

Two important conclusions that can be drawn from the results of experiments described in this paper are: (I) closed vesicular structure of the inner membrane demanded by the chemiosmotic hypothesis (5) is not essential for oxidative phosphorylation and (II) the mechanism of uncoupling by a classical uncoupler is different from the mechanism of uncoupling by the ionophores in the presence of K^+. Since exposure to lysolecithin, a reagent that disrupts lipid bilayers, did not diminish the sensitivity of ETP to FCCP, it appears very likely that classical uncouplers such as FCCP exert their uncoupling effect through interaction with a protein component of the inner membrane (6) rather than by transporting protons through the lipid bilayers (7). This conclusion is supported by the results of studies on respiratory control in cyto-

chrome oxidase vesicles (8). In this system that contained purified cytochrome oxidase and lipid, the ionophores were very effective in releasing respiratory control while classical uncouplers in the absence of valinomycin were much less effective presumably due to the absence of the protein responsible for uncoupler binding.

ACKNOWLEDGEMENTS

The authors wish to express their thanks to Dr. David E. Green for his helpful discussions during the course of this work. Robert A. Haworth is grateful to the Wellcome Trustees for the award of a Wellcome Research Travel Grant. We are grateful to Ms. J. Hurley for her expert technical assistance. Meat by-products were generously supplied by Oscar Mayer and Co. This investigation was supported in part by the National Institute of General Medical Sciences, Program Project Grant GM-21847.

REFERENCES

1. Komai, H., Hunter, D. R. and Takahashi, Y., Biochem. Biophys. Res. Commun., 53, 1 (1973).
2. Lindberg, O. and Ernster, L. in Glick, D. (ed.) Methods in Biochemical Analysis 3, Interscience, New York (1956) p. 1.
3. Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J., J. Biol. Chem., 193, 265 (1951).
4. Honjo, I. and Ozawa, K., Biochim. Biophys. Acta, 162, 624 (1968).
5. Mitchell, P., Chemiosmotic Coupling and Energy Transduction, Glynn Research Bodmin, Cornwall (1968).
6. Hatefi, Y., Hanstein, W. G., Davis, K. A. and You, K. S., Ann. N.Y. Acad. Sci., 277 (in press).
7. Bakker, E. P., Van Den Heuvel, E. J., Wiechmann, A.M.C.A., and Van Dam, K., Biochim. Biophys. Acta, 292, 78 (1973).
8. Hunter, D. R. and Capaldi, R. A., manuscript in preparation.