COMPETITION BETWEEN OXIDATIVE PHOSPHORYLATION AND ENERGY-LINKED PYRIDINE NUCLEOTIDE TRANSHYDROGENATION IN SUBMITOCHONDRIAL PARTICLES

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Previous work in this laboratory (Danielson and Ernster, 1963; Lee and Ernster, 1964, 1966) has led to the conclusion that the energy-linked reduction of $NADP^+$ by NADH catalyzed by submitochondrial particles from rat liver or beef heart is driven by a high-energy intermediate of the respiratory chain-linked oxidative phosphorylation system. The following reaction sequence has been postulated:

$$NADH + NADP^+ + I\sim X \longrightarrow NAD^+ + NADPH + I + X \quad (1)$$

where I~X is a high-energy intermediate which can be generated either by the respiratory chain:

$$I + X \xrightarrow{\text{respiration}} I\sim X \quad (2)$$

or by ATP

$$ATP + I + X \longleftrightarrow ADP + P_i + I\sim X \quad (3)$$

It follows from the above reaction sequence that the energy-linked pyridine nucleotide transhydrogenation and oxidative phosphorylation are in a competitive relationship with regard to I~X generated by the respiratory chain. Recently, van Dam and ter Welle (1966) and van Dam (1966) have studied the two processes as occurring separately or together in sub-

mitochondrial particles from beef heart, and observed unaltered efficiencies of phosphorylation (P/O) and NADH-linked $NADP^+$ reduction ($NADP^+/O$) coupled to succinate oxidation. They have further found that inhibition of succinate oxidation by increasing concentrations of malonate did not effect the P/O ratio but markedly increased the $NADP^+/O$ ratio, although the net rate of $NADP^+$ reduction was decreased, in accordance with out previous results (Lee et al., 1964). They have concluded that there is no competition between oxidative phosphorylation and NADH-linked $NADP^+$ reduction coupled to succinate oxidation. In this paper, we wish to report data which demonstrate such a competition, resulting in a substantial reduction of P/O ratio by the energy-linked pyridine nucleotide transhydrogenase reaction.

The experiments were performed with submitochondrial particles prepared after sonication of beef-heart mitochondria in the presence of Mg^{++} and ATP (Löw and Vallin, 1963). Suspensions of the particles were incubated in a tris-acetate-buffered sucrose medium, in the chamber of a Clark O_2 electrode. P_i^{32}, ADP, Mg^{++}, hexokinase, glucose, succinate, rotenone, NADH, $NADP^+$, and malonate were added as specified in the table and figure legends. Respiration was monitored until about two-thirds of the oxygen was consumed, at which time the sample was rapidly fixed by the addition of 0.3 ml 5 M H_2SO_4. An aliquot of the sample was used for determination of P_i uptake by the isotope distribution method of Lindberg and Ernster (1955). The remainder of the sample was neutralized, centrifuged, and an aliquot of it was used for determining $NADP^+$ enzymically with glucose-6-phosphate and glucose-6-phosphate dehydrogenase (Klingenberg, 1962).

Table I

Efficiencies of phosphorylation and energy-linked pyridine nucleotide transhydrogenation coupled to the aerobic oxidation of succinate.

particle protein mg/assay	Additions	O_2 mμatoms/ min	P_i mμmoles/ min	$NADP^+$ x mμmoles/ min	P/O	$NADP^+/O$	Σ (P/O+$NADP^+$/O)
0.45	-	155	110	-	0.71	-	0.71
0.45	NADH+$NADP^+$	144	78	50	0.54	0.35	0.89
0.45	NADH	149	97	-	0.65	-	0.65
0.45	$NADP^+$	157	110	-	0.70	-	0.70
0.45	NADH+$NADP^+$-P_i	131	-	95	-	0.73	0.73
0.45	NADH+$NADP^+$+olig.	122	5	101	0.04	0.83	0.87
3.0	Malonate (Malon.)	188	126	-	0.67	-	0.67
3.0	Malon.+NADH+$NADP^+$	178	46	203	0.26	1.15	1.41
3.0	Malon.+NADH	178	106	-	0.60	-	0.60
3.0	Malon+$NADP^+$	161	105	-	0.65	-	0.65
3.0	Malon.+NADH+$NADP^+$ -P_i	168	-	234	-	1.39	1.39
3.0	Malon.+NADH+ $NADP^+$+olig.	175	8	250	0.04	1.43	1.47

[x] corrected for the non-energy-linked transhydrogenase activity.

The reaction mixture consisted of 180 mM sucrose, 50 mM tris-acetate buffer, pH 7.5, 3 mM P_i (1.2×10^6 cpm/μmole), 10 mM $MgSO_4$, 2 mM ADP, 60 mM glucose, 75 units hexokinase, 3.3 μM rotenone, and 3.3 mM succinate, and when indicated, 2 mM NADH, 0.5 mM semicarbazide, 100 μg alcohol dehydrogenase, 120 mM ethanol, 2 mM $NADP^+$, 0.66 mM malonate and 5 μg oligomycin. Final volume, 3 ml; temperature, 30°C.

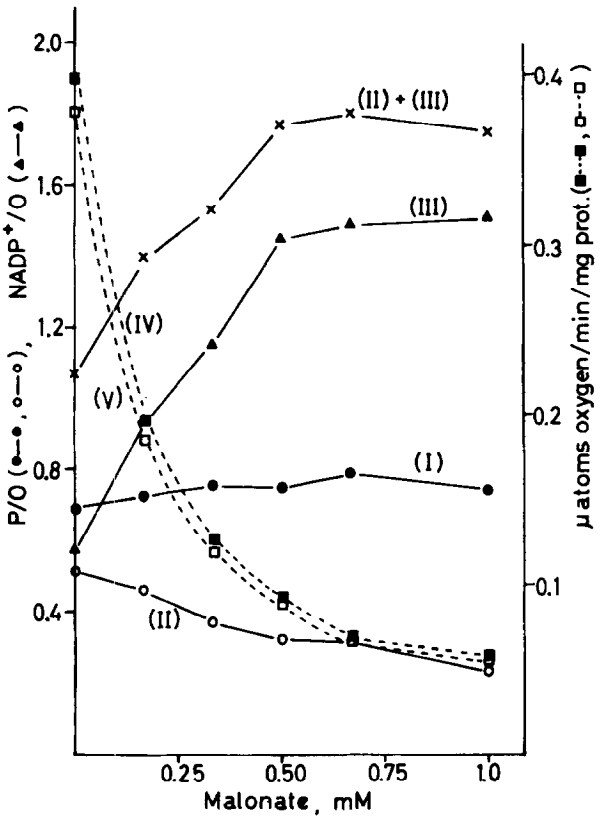

Figure 1. Effect of malonate on P/O and $NADP^+$/O ratios.

Conditions were as in Table 1 except that 0.60 mg instead of 0.45 mg particle protein was present in the absence of malonate.

Curve I: P/O ratio in absence of NADH + $NADP^+$;

Curves II and III: P/O ratio and $NADP^+$/O ratio, respectively, in presence of NADH + $NADP^+$;

Curves IV and V: oxygen uptake in absence and presence, respectively, of NADH + $NADP^+$.

The results of a typical experiment are summarized in Table I. When the energy for the reduction of $NADP^+$ by NADH was supplied by the aerobic oxidation of succinate, there occurred a low rate of $NADP^+$ reduction, and the extent of phosphorylation coupled to succinate oxidation was only slightly influenced by the energy-linked transhydrogenase reaction. The sum of the P/O and $NADP^+$/O ratios was 0.89, indicating that, taking 2 as the maximal ~/O ratio for the aerobic oxidation of succinate, the efficiency of the system was about 45%. When a larger amount of enzyme was employed, and the rate of succinate oxidation was suppressed by the addition of malonate, the $NADP^+$/O ratio increased markedly, in line with the findings of van Dam and ter Welle (1966) and van Dam (1966), but, in contrast with their results, the P/O ratio simultaneously decreased.

The sum of the $NADP^+$/O and P/O ratios was 1.41 in this particular experiment, but could reach a value of 1.8 (cf. Figure 1), i.e., an efficiency of 90%. Little or no effect was observed with either NADH or $NADP^+$ alone. Thus, an active transhydrogenase reaction seems to be necessary for the observed decrease in P/O ratio. Abolition of phosphorylation by either omission of phosphate or addition of oligomycin raised the $NADP^+$/O ratio as expected. The effect of increasing concentrations of malonate is shown in Figure 1.

The kinetics of the competition between oxidative phosphorylation and energy-linked transhydrogenation was investigated by varying the concentrations of the reactants of the two systems, i.e., NADH or $NADP^+$ and P_i or ADP, respectively. As expected, lack of saturation of the transhydrogenase system with NADH and/or $NADP^+$ lowered, and lack of saturation of the phosphorylating system with P_i and/or ADP increased, the extent of competition in favor of the transhydrogenase reaction. Unexpectedly, however, it was found that the saturating concentration of NADH for the energy-linked transhydrogenase reaction was higher in the presence than in the absence of an active phosphorylating system; and conversely, that the saturating concentration of P_i for oxidative phosphorylation was higher in the presence than in the absence of an active transhydrogenase system. These findings, which are now under further exploration, may explain the previous failure of van Dam and ter Welle (1966) and van Dam (1966) to observe a competition between energy-linked transhydrogenation and oxidative phosphorylation under the conditions of their experiments.

In summary, the present data demonstrate that energy-linked reduction of $NADP^+$ by NADH efficiently competes with

oxidative phosphorylation coupled to the aerobic oxidation of succinate in submitochondrial particles from beef heart. These results are in good accordance with the concept (Danielson and Ernster, 1963; Lee and Ernster, 1966) that the energy-linked transhydrogenase reaction derives energy from an intermediate of the respiratory chain-linked phosphorylation system, and may constitute a valuable basis for future investigations into the chemical nature of this intermediate.

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REFERENCES

Danielson, L., and Ernster, L., Biochem. Biophys. Res. Commun., 10, 91 (1963); in Johnson Foundation Colloquium on Energy-Linked Functions of Mitochondria, Philadelphia, 1963. B. Chance, ed., Academic Press, New York, 1963, p. 157; Biochem. Z., 338, 188 (1963).

Klingenberg, M., in Methoden der Enzymatischen Analyse, H.-U. Bergmeyer, ed., Verlag Chemie, Weinheim, 1962, p. 535.

Lee, C.P., and Ernster, L., Biochim. Biophys. Acta, 81, 187 (1964).

Lee, C.P., Azzone, G.F., and Ernster, L., Nature, 201, 152 (1964).

Lee, C.P., and Ernster, L., in Symp. on Regulation of Metabolic Processes in Mitochondria, Bari, 1965. J.M. Tager, S. Papa, E. Quagliariello, and E.C. Slater, eds., Elsevier, Amsterdam, B.B.A. Library, 7, 218 (1966).

Lindberg, O., and Ernster, L., Meth. Biochem. Anal., 3, 1 (1955).

Löw, H., and Vallin, I., Biochim. Biophys. Acta, 69, 361 (1963).

van Dam, K., Ph.D. Thesis, University of Amsterdam, 1966.

van Dam, K., and ter Welle, H.F., in Symp. on Regulation of Metabolic Processes in Mitochondria, Bari, 1965. J.M. Tager, S. Papa, E. Quagliariello, and E.C. Slater, eds., Elsevier, Amsterdam, B.B.A. Library, 7, 237 (1966).