COPPER-DEFICIENT MITOCHONDRIA
ELECTRON TRANSPORT AND OXIDATIVE PHOSPHORYLATION

Hartmut Wohlrab and Earl E. Jacobs
Biophysics Laboratory, Stanford University, Stanford, California 94305

Received August 14, 1967

Some limited studies on the capacity of copper-deficient rat liver mitochondria to sustain electron transport and oxidative phosphorylation have been carried out by Gallagher et al. (1956). These authors observed that the decrease in succinoxidase activity was not commensurate with the decrease in cytochromes a-a_3, without becoming aware of the possible relation of this anomaly to interchain electron transport. Holmes (1960), Chance (1964, 1965) and Estabrook (1965) conducted detailed spectral kinetic studies of the interaction of coupled and uncoupled respiratory chains which receive their electrons from the same substrate. The interchain electron transport rates were generally found to be considerably slower than those for transport along a single cytochrome chain. The experiments described in this communication indicate that interchain electron transport plays a significant role in the respiratory reactions of mitochondria lacking a normal complement of cytochrome oxidase as a consequence of copper-deficiency.

MATERIALS AND METHODS

The mitochondria were prepared from the livers of normal and copper-deficient rats by homogenization and differential centrifugation in 0.25 M sucrose, according to the procedure described by Lardy and Wellman (1952). These preparations were tightly coupled, exhibiting typical respiratory control ratios. Such mitochondria were washed once with distilled water to produce preparations lacking respiratory control, and then twice with

0.15 M KCl to produce preparations depleted of endogenous cytochrome c . Various substrates and electron donors were used as indicated to initiate the electron transport process at selected points along the chain. P:O ratios were calculated directly from the ADP:ΔO ratios, as described by Chance and Williams (1955). Oxygen consumption was measured with a Clark electrode covered with a 0.5 mil teflon membrane; the reaction medium was stirred rapidly to insure that the measured rates were not diffusion-limited.

TABLE I

RESPIRATORY PROCESSES IN NORMAL AND COPPER-DEFICIENT, COUPLED, RAT LIVER MITOCHONDRIA

PREPARATION	Cu(-)I	Cu(-)II	Cu(-)III	Cu(+)I	Cu(+)II	Cu(+)III
Cyt a-a_3)[1]	.09	.08	.11	.45	.45	.45
Succinate[2]	.96	.89	1.08	1.45	1.47	1.27
% Δ + cyt c)[3]	10	-	6	8	1	23
P:O[4]	1.60	1.58	1.43	1.78	1.84	1.50
	1.55	1.63	1.49	1.60	1.78	1.60
R.C. Ratio[5]	3.7	4.8	5.7	5.6	6.0	5.0
	2.9	3.5	4.2	5.3	5.5	3.9
Glutamate[6]	.45	.45	.43	.41	.48	.38
P:O	2.29	2.32	2.09	2.22	2.37	2.24
R.C. Ratio	4.1	4.0	11.6	3.4	8.0	5.0
	5.0	-	3.8	3.9	-	5.0
ASC-TMPD[7]	1.84	1.42	2.17	3.00	2.88	3.00
% Δ + cyt c	4	-	7	8	-	12

[1] ΔO.D. 605-630 mμ, dithionite reduced minus oxidized, of 20 mg protein per ml, solubilized in 6 percent Triton X 100 and .16 M KPO_4 , pH 7.0.

[2] mμ atoms oxygen consumed/min/mg. protein, in 10 ml total volume of reaction medium containing 1.25 mg protein/ml, ADP for state 3 respiration, .25M sucrose, 10mM neutral potassium phosphate, 7.5mM $MgCl_2$, 1mM EDTA, and 7.5mM potassium succinate.

[3] Percent change of state 3 respiration on adding 1 mg cytochrome c.

[4] P:O ratio, measured polarographically (Reference 1).

[5] Respiratory control ratio.

[6] Basic reaction system same as [2] except substrate is 7.5mM K glutamate.

[7] Basic reaction system same as [2] except substrate is 7.5mM K ascorbate plus 1mM tetramethylphenylenediamine.

RESULTS

The data of Table I show typical respiration rates and P:O ratios for both the normal and copper-deficient, tightly coupled, rat liver mitochondria. Both the P:O ratios and the respiratory control ratios characteristic of succinate oxidation appear to be slightly lower in the copper-deficient mitochondria than in the normal mitochondria. However, the ratios characteristic of glutamate oxidation appear to be the same in both preparations. Stimulation by added cytochrome c of state 3 respiration, initiated by either succinate or ascorbate-TMPD, was also about the same in both preparations. The data of Table II show typical respiration rates for the water-washed mitochondria. Stimulation of succinate oxidation by added cytochrome c now appears to be somewhat greater in the copper-deficient preparation than in the normal one. The data of Table III show typical reaction rates for the mitochondrial preparations depleted of endogenous cytochrome c by water-salt washings. Attention is drawn to the close agreement between the relative decrease of cytochrome oxidase content as determined spectroscopically, and that of the ascorbate-TCBQ-polylysine reaction rate. Table IV compares the percent decrease in the various respiration processes of the normal and copper-deficient preparations.

TABLE II

RESPIRATION RATES OF WATER-WASHED,
NORMAL AND COPPER-DEFICIENT RAT LIVER MITOCHONDRIA

PREPARATION	Cu(-)I	Cu(-)III	Cu(+)I	Cu(+)III
Succinate[1]	.65	.60	1.15	1.22
% Δ + cyt c	22	85	12	0
ASC-TMPD[1]	1.27	1.20	2.70	2.78
% Δ + cyt c	135	175	165	175
ASC-CYT c[2]	.31	.31	.27	.36

[1] Basic reaction system as in Table I except ADP omitted.
[2] Basic reaction system as in [1] except substrate is 7.5 mM K ascorbate plus 1 mg cyt c.

TABLE III

RESPIRATION RATE OF RAT LIVER MITOCHONDRIA
DEPLETED OF ENDOGENOUS CYTOCHROME c BY WATER-SALT WASHING

PREPARATION	Cu(-)III	Cu(+)I
ASC-TCBQ-Polylysine[1]	0.58	1.89

[1]Basic reaction system as in Table I except substrate is 7.5mM K ascorbate + 1mM tetrachlorobenzoquinol (TCBQ) + 1 mg polylysine.

TABLE IV

PERCENT DECREASE IN RESPIRATION RATES OF COPPER-DEFICIENT, COMPARED TO NORMAL, RAT LIVER MITOCHONDRIAL PREPARATIONS

%(Δ-) Cytochrome a-a_3	78
%(Δ-) Succinate (C)[1]	37
%(Δ-) Succinate (W)	46
%(Δ-) Glutamate (C)	0
%(Δ-) ASC-TMPD (C)	48
%(Δ-) ASC-TMPD (W)	54
%(Δ-) ASC-TCBQ (W-S)	69

[1](C) is coupled mitochondria; (W) is water-washed; (W-S) is water and salt washed.

DISCUSSION

Although the conclusions to be drawn from the data presented in this communication must be considered as only tentative, it seems fairly evident that the further from the oxygen reduction site that electrons are injected into the respiratory chain, the greater is the contribution of interchain electron transport to the observed reaction rate. Water-washed mitochondria appear to have less interchain contributions to the overall rates than do the coupled mitochondria. This may be a consequence of the structural disruption of the mitochondria induced by the hypotonic exposure. Chance (1967) found that mitochondria treated with uncoupling agents show higher interchain

electron transport rates than do coupled mitochondria. It is to be emphasized that one possible physiological significance of interchain electron transport is its potential capacity to raise respiration rates by 100 percent or more. The phenomenon could alternately be viewed as a physiological protection of the respiratory processes against terminal inhibition of the electron transport chain.

REFERENCES

1. Chance, B., and Williams, G. R., J. Biol. Chem. 217, 383 (1955).
2. Chance, B., Abstracts, 6th Int. Cong. Biochem., p. 617, N.Y.C. (1964).
3. Chance, B., in "Oxidases and Related Redox Systems", edited by T. King, H. Mason and M. Morrison, Vol. II, 929, John Wiley and Sons (1965).
4. Chance, B., Abstracts, 51 National Meeting, F.A.S.E.B., p. 609, Chicago (1967).
5. Estabrook, R., in "Oxidases and Related Redox Systems", edited by T. King, H. Mason and M. Morrison, Vol. II, 939, John Wiley and Sons (1965).
6. Gallagher, C. H., Judah, J. D., and Rees, K. R., Proc. Roy. Soc. Lon., B145, 134 (1956).
7. Holmes, W. F., Dissertation, Univ. of Penn. (1960).
8. Lardy, H. A., and Wellman, H., J. Biol. Chem., 195, 215 (1952).

ACKNOWLEDGEMENTS

This research was supported by Grant G 64-31 from the Life Insurance Medical Research Fund, Grant HE-07103 from the U. S. Public Health Service and a predoctoral USPHS fellowship to Hartmut Wohlrab.