Biochimica et Biophysica Acta, 292 (1973) 88–91

BBA 46448

STUDIES OF OXIDATIVE PHOSPHORYLATION IN *SACCHAROMYCES CEREVISIAE* AND *SACCHAROMYCES CARLSBERGENSIS*

BRUCE MACKLER and BYRON HAYNES
Department of Pediatrics, University of Washington, Seattle, Wash. (U.S.A.)
(Received August 28th, 1972)

SUMMARY

Mitochondria isolated from cultures of *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis*, which were harvested late in the stationary phase of growth at a time when full development of the electron transport and associated phosphorylation systems had occurred, carried out oxidative phosphorylation with P:O ratios of approximately 3 and 2 when pyruvate–malate and succinate, respectively, were used as substrates.

Recently, Ohnishi[1] has reported the finding of three sites of phosphorylation with NAD-linked substrates in cultures of *Saccharomyces carlsbergensis* which were aerated in phosphate buffer after having reached the early stationary phase of growth. Previously, reports by other workers have differed in their findings and P:O ratios of both 2 and 3 have been described[2–4], suggesting to our laboratory that the difference in results might be a reflection of the phase of growth and degree of maturity of the yeast at the time of harvest and that the presence of a third site of phosphorylation represents normal full development of the phosphorylation systems and not a specific induction as suggested by Ohnishi[1].

Cultures of *Saccharomyces carlsbergensis* (ATCC No. 9080) and *Saccharomyces cerevisiae* (Fleischmann) were kindly given us by Dr Howard Douglas of the Department of Microbiology of the University of Washington. The yeasts were grown in 10-l batches in a medium containing 1% dextrose, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.1% $(NH_4)_2SO_4$, 0.2% Difco yeast extract and 0.1% sodium acetate in a New Brunswick Fermacell Fermentor at 30 °C with rapid stirring and aeration of 1 ft^3/min. The yeasts were harvested by centrifugation either in late stationary phase of growth (between 5 and 7 h after they had entered the stationary phase) or in early stationary phase of growth (less than 1 h after entering the stationary phase), were washed once with distilled water, and mitochondria were prepared as follows. Approximately 10 g (wet wt) of yeast were washed by centrifugation with a solution containing 0.5 M mannitol, 0.4% bovine serum albumin and 0.1 mM disodium ethylenedinitrilotetraacetate (versene) of pH 7.0 and the cells were resuspended in the same solution to a concentration of 1 g (wet wt) per 3.5 ml. 30 ml of the yeast mixture were mixed with 35 g of fine glass beads[5] and the mixture was shaken for 8 s with cooling in a Bronwill model MSK cell homogenizer. All subsequent procedures were carried out at 0–5 °C. The homogenate was decanted from the glass

beads and centrifuged at $2000 \times g$ for 10 min. The cloudy supernatant was removed and recentrifuged for 10 min at $8000 \times g$. The residue containing the mitochondria was suspended in a solution of 0.5 M mannitol and 0.4% bovine serum albumin of pH 7.0 (approximately 10 ml), and the suspension was again centrifuged at $2000 \times g$ and $8000 \times g$. The final residue (mitochondrial preparation) was suspended again in the mannitol–bovine serum albumin solution by gentle homogenization.

Studies of oxidative phosphorylation were carried out in a Warburg apparatus by standard techniques. The main compartment of the Warburg vessel contained the following: 0.15 ml of a solution (pH 6.8) containing 0.15 M KH_2PO_4, 0.1 M Tris and 0.01 M versene; 0.075 ml of 0.1 M $MgCl_2$ solution; 0.15 ml of 5% bovine serum albumin solution; 0.15 ml of 1% hexokinase solution; 0.5 ml of 0.75 M mannitol solution; and 0.22 ml of mitochondrial suspension (approximately 2.5 mg protein). The side arm of the vessel contained 0.025 ml of 0.1 M ADP solution, 0.015 ml of 1 M glucose and 0.03 ml of either molar succinate or pyruvate–malate (9:1, by vol.) solution of pH 7.2. A small wick of filter paper and 0.2 ml of 20% KOH solution were placed in the center well of the flask. The vessels were allowed to equilibrate at 26 °C for 7 min, the stopcocks of the manometers were closed and the side arm contents tipped into the main compartment. Oxygen uptake was measured for 12–15 min, at which time the reactions were terminated by addition of trichloroacetic acid to a final concentration of 5%. Inorganic phosphate was determined in duplicate in the assay mixtures, before and after the reaction, by the Fiske–Subba-Row method[6], and the difference represented the amount esterified to form ATP.

Protein was determined by the method of Lowry *et al.*[7]. ADP and hexokinase were obtained from Sigma Chemical Company.

Mitochondria, prepared as described above from yeast harvested in the late stationary phase of growth, were studied for their ability to carry out phosphorylation and the results are shown in Table I. As shown in the table, mitochondria from both *S. cerevisiae* and *S. carlsbergensis* carried out oxidative phosphorylation with P:O ratios of approximately 3 and 2 when pyruvate–malate and succinate, respectively, were used as substrates.

In other experiments mitochondria prepared from yeast harvested in early stationary phase of growth were studied for their ability to carry out oxidative phosphorylation and the results are shown in Table II. As shown in the Table, the P:O ratios for mitochondria from *S. cerevisiae* and *S. carlsbergensis* were lower than for the mitochondria from yeasts harvested in the late stationary growth phase (see Table I) and were approximately 2 for pyruvate–malate as substrate and slightly lower for succinate.

Finally, P:O ratios were determined with pyruvate and malate as substrate for mitochondria isolated from *S. cerevisiae* harvested in the late stationary phase of growth using a Clark oxygen electrode to measure oxygen consumption, and ^{33}P incorporation into ATP to measure ATP formation[8], and the results were in good agreement with those determined manometrically with P:O ratios above 2.5 in 3 experiments (average 2.6). The composition of the assay medium was the same as used in the manometric assays.

The results detailed above confirm the previous findings of Ohnishi[1] for *S. carlsbergensis*, and demonstrate that both species of yeast possess the ability to phosphorylate at three sites with NAD-linked substrates when growth conditions

TABLE I

RESULTS OF EXPERIMENTS OF OXIDATIVE PHOSPHORYLATION WITH *S. CEREVISIAE* AND *S. CARLSBERGENSIS*

All results are the average of duplicate experiments.

Substrate	*S. cerevisiae*			*S. carlsbergensis*		
	μmoles P esterified	*μatoms O_2 utilized*	*P:O ratio*	*μmoles P esterified*	*μatoms O_2 utilized*	*P:O ratio*
Succinate	4.4	2.4	1.8	3.6	1.9	1.9
	5.0	2.7	1.9	4.3	2.4	1.8
	5.0	3.0	1.7	4.2	2.6	1.6
	5.7	3.0	1.9	4.2	2.5	1.7
	5.0	2.5	2.0	4.7	2.4	2.0
	5.2	2.3	2.3	5.5	2.6	2.1
Average	5.0	2.7	1.9	4.4	2.4	1.9
Pyruvate–malate	3.5	1.1	3.2	5.4	1.9	2.8
	4.1	1.4	2.9	4.0	1.4	2.9
	5.4	2.0	2.7	4.7	1.9	2.5
	6.0	2.1	2.9	4.9	1.7	2.9
	5.7	2.2	2.6	5.8	2.2	2.6
	5.4	1.7	3.2	5.8	1.9	3.1
Average	5.0	1.8	2.8	5.1	1.8	2.8

TABLE II

EXPERIMENTS OF OXIDATIVE PHOSPHORYLATION WITH YEAST HARVESTED IN EARLY STATIONARY GROWTH PHASE

All results are the average of duplicate experiments.

Substrate	*S. cerevisiae*			*S. carlsbergensis*		
	μmoles P esterified	*μatoms O_2 utilized*	*P:O ratio*	*μmoles P esterified*	*μatoms O_2 utilized*	*P:O ratio*
Succinate	4.9	3.3	1.5	2.5	2.1	1.2
	3.5	2.9	1.2	5.2	3.5	1.5
	5.6	4.0	1.4	4.8	2.7	1.8
	5.4	3.4	1.6	5.5	3.6	1.5
	6.4	3.2	2.0	4.2	2.2	1.9
Average	5.2	3.4	1.5	4.4	2.8	1.6
Pyruvate–malate	2.5	1.2	2.1	2.7	1.6	1.7
	2.7	1.2	2.3	5.2	2.9	1.8
	6.0	3.0	2.0	4.9	2.1	2.3
	5.6	2.5	2.2	5.1	2.5	2.0
	4.8	2.3	2.1	4.0	2.0	2.0
Average	4.3	2.0	2.1	4.4	2.2	2.0

favor the full development of the electron transport and associated phosphorylation systems. In previous work[5,9] we have described the isolation of purified components of the electron transport systems of *S. cerevisiae* (of the same strain as used in this report) and have shown the systems to differ from the electron transport systems of heart muscle in several ways: namely, the electron transport particle from *S. cerevisiae* is not amytal or seconal sensitive, contains FAD and not FMN as the prosthetic group of the NADH dehydrogenase, and does not appear to contain a nonheme iron, labile sulfide component in the NADH dehydrogenase region of the enzyme. Since all sites of phosphorylation appear to be present in *S. cerevisiae*, despite the differences in structure from heart electron transport particles mentioned above, it would appear that these structural differences are not of importance to the operation of phosphorylation in the NADH dehydrogenase region of the mitochondrial systems, and that the previous findings reported by other workers[10,11] of only two sites of phosphorylation for *S. cerevisiae* with NAD-linked substrates were due to premature harvesting of the yeast before full development of the phosphorylation systems had occurred.

ACKNOWLEDGMENTS

The authors wish to thank Dr Howard Douglas for providing us with the parent strains of yeast, and for checking the strains to ensure their identity.

This study was supported in part by grants HE-05457 and HD-05961 from the National Institutes of Health.

REFERENCES

1 Ohnishi, T. (1970) *Biochem. Biophys. Res. Commun.* 41, 344–352
2 Ohnishi, T., Kawaguchi, K. and Hagihara, B. (1966) *J. Biol. Chem.* 241, 1797–1806
3 Schuurmans-Steckhoven, F. M. A. H. (1966) *Arch. Biochem. Biophys.* 115, 555–568
4 Ghosh, A. and Bhattacharyya, S. N. (1971) *Biochim. Biophys. Acta* 245, 335–346
5 Mackler, B., Collipp, P. J., Duncan, H. M., Rao, N. A. and Huennekens, F. M. (1962) *J. Biol. Chem.* 237, 2968–2974
6 Fiske, C. H. and SubbaRow, Y. (1925) *J. Biol. Chem.* 66, 375–400
7 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* 193, 265–275
8 Nielsen, S. O. and Lehninger, A. L. (1955) *J. Biol. Chem.* 215, 555–570
9 Duncan, H. M. and Mackler, B. (1966) *Biochemistry* 5, 45–50
10 Balcavage, W. X. and Mattoon, J. R. (1968) *Biochim. Biophys. Acta* 153, 521–530
11 Kovac, L. (1968) *Biochim. Biophys. Acta* 153, 32–42