Vol. 97, No. 1, 1980
November 17, 1980

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 263-269

ASSEMBLY OF SUCCINIC DEHYDROGENASE COMPLEX INTO MITOCHONDRIAL MEMBRANE IN YEAST

Javed Ashraf, T.Somasundaram and J.Jayaraman*

Department of Biochemistry, School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, India

Received October 1,1980

Summary

In yeast cells subjected to glucose repression, the succinic dehydrogenase complex is found to be solubilised into the cytosol, and during derepression, the soluble activity is reintegrated into the membrane. This reintegration has been shown under *in vitro* conditions also. Membrane fractions from cells treated with chloramphenicol (CAP) and not cycloheximide (CHI) fail to integrate the soluble enzyme, indicating the need for a mitochondrially synthesized product. Preliminary evidence presented here implicate phospholipids, particularly cardiolipin, for an anchoring role in the membrane.

Introduction

Glucose repression of mitochondriogenesis and the subsequent derepression in yeast (*Saccharomyces cerevisiae*) cells have been extensively used in this laboratory to understand the process of mitochondriogenesis (1-5). In this communication we show that during glucose repression, the succinic dehydrogenase is solubilised into the cytosol. This solubilised enzyme is reintegrated into the membrane, *in vivo*, during derepression and *in vitro* by incubation with appropriate membranes. Results presented also show that cardiolipin might have a specific role in this integration process.

Publication No.037 from the Department of Biochemistry

*to whom correspondence should be addressed

CAP - Chloramphenicol; DCPIP - 2,6-dichloroindophenol; CHI - Cycloheximide; CL - Cardiolipin; PC - Lecithin; PL - Total phospholipids

0006-291X/80/210263-07$01.00/0

Materials and Methods

The culturing of the yeast (Saccharomyces cerevisiae 3095), induction of repression and derepression, preparation of mitochondrial particles, have all been described earlier (1). Succinic dehydrogenase activity was measured following the methods of Green et al (16). The assay mixture consisted of succinate (0.05 ml, 33 mM) azide (0.05 ml, 33 mM), DCPIP (0.06 ml, 0.01%), phenazine methosulfate (0.6 ml, 0.01%) and approximately 100 µg protein. The total volume was made upto 1 ml with the isolation buffer. The rate of reduction of the dye at 600 nm was followed and the activities are expressed as nmol of DCPIP reduced per minute per mg protein (specific activity) or as nmole of DCPIP reduced per minute per 50 ml of cell suspension (total activity).

Protein was estimated by the method of Lowry et al (7). Standard procedures were used to extract and estimate phospholipids (8-11). Cardiolipin was purified using the technique developed by Murugesh and Jayaraman (12). Fresh sonicated lipid preparations were used in all the cases. The isolation buffer used was 0.1 M phosphate buffer (pH 7.4) containing 50 mM succinate and this was found to keep the enzyme activity stable for more than 10 hrs.

Results and Discussion

Fig.1 shows the variations in the succinic dehydrogenase activities in the mitochondrial pellet as well as the post-mitochondrial supernatant fractions, during the various stages of repression and derepression.

During repression, i.e. the first 2½ hrs, the total as well as specific activities in the mitochondrial fractions decreased by 4 and 3.1 fold respectively. Subsequently during derepression, the activities in the mitochondrial pellet increased in parallel with the other enzyme activities reported earlier (5). The total activity (mitochondrial plus the supernatant fraction) remained constant during the first 1½ hrs and between 1½ and 2½ hrs showed an increase of 1.4 fold, at which level it remained during the derepression.

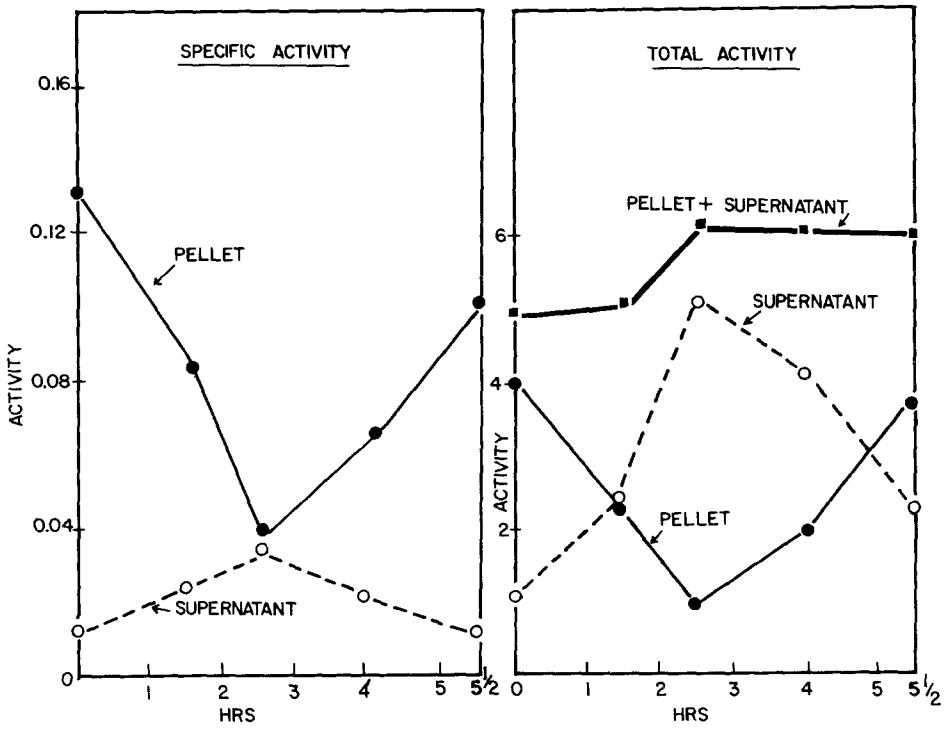

Fig.1

Cells were inoculated (4 mg per ml) in the repression medium, then harvested at various times and mitochondria were isolated. Total activity is expressed as μmole of DCPIP reduced per 50 ml of cell suspension. Specific activity is expressed as μmol of DCPIP reduced per minute per mg protein. The time interval between 0 to 2½ hrs represents the repression phase, while that between 2½ to 5½ hrs represents derepression phase.

● ——— ● mitochondrial pellet

O ----- O post mitochondrial supernatant

The results suggest: i. that during repression the enzyme is released into the cytosol. The rate of turnover is slow. ii. The solubilised enzyme along with some newly synthesized enzyme is integrated into the membrane during derepression (see also Table 1, where it is shown that treatment of cells with cycloheximide reduces the enzyme activity only by 40% -

Table 1

Pellet		Cytosol	Expected activity	Observed	% increase	Additions	Expected activity	Observed	% increase
R		-	--	20	--	CL+	20	21	5
R	+	R	20	19	--	CL	20	30	50
			25	22	--	PC	25	28	12
			20	20	--	PL	20	23	15
D		-	--	88	--	CL	88	93	6
		-	--	92	--	PC	92	94	2
		-	--	92	--	PL	92	92	0
D	+	R	88	121	37	CL	88	126	43
		-	92	124	35	PC	92	126	38
		-	92	124	35	PL	92	118	28
R		-	--	35	--	--	--	--	--
R		R	35	32.5	--	--	--	--	--
R_{CHI}		-	--	42	--	--	--	--	--
R_{CHI}		R	42	66	58	--	--	--	--
R_{CAP}		-	--	55	--	--	--	--	--
R_{CAP}		R	55	57	4	CL	55	70	27
						PC	55	59	7

R - Repressed; D - partially derepressed; R_{CHI} - CHI treated; R_{CAP} - CAP treated;
CL- Cardiolipin; PC - Lecithin; PL - Total phospholipid

Activity is expressed as nmol of DCPIP reduced/min/mg protein. Expected values represent the activity if there is no migration of activity to the pellet (calculated on the basis of activity of the incubated pellet)

+ The Cardiolipin : protein ratio was maintained to approximately (1:2) in all the cases. The optimal stimulatory concentration of cardiolipin was found to be half the amount of protein used (Data not given)

50% under conditions where synthesis of ATPase and cytochrome oxidase is blocked to about 90% (data not given).

In order to verify whether this integration can be achieved under *in vitro* conditions, the following experiments were carried out. Mitochondrial pellets from repressed (2½ hrs) and partially derepressed (5½ hrs) cells were incubated with the supernatant fraction of the repressed cells which contains the maximum enzyme activity in the soluble state. After incubation the pellets were isolated and assayed for enzyme activity. The data on the 'expected values' calculated, and the 'observed values', for different mixtures are given in Table 1. Under the experimental conditions, any increase of the 'observed values' over the 'expected values', is a measure of the migration of the soluble enzyme complex to the membrane.

These results show that: a. the soluble enzyme does not integrate with the mitochondrial pellet obtained from repressed cells, but integrates with membrane fractions from partially derepressed cells, and b. treatment of the cells with chloramphenicol at the start of derepression (2½ hrs) prevents the membrane fraction from integrating the soluble activity.

While the above results suggest that mitochondrial protein synthesis is necessary for the integration, so far a direct involvement of a mitochondrial protein in the expression of the enzyme activity has not been demonstrated. Preliminary results indicate that cardiolipin could be involved. That

cardiolipin could be a factor in this integration was considered for the following reasons: a. Lipids are essential for maintaining the membrane structure (13); b. Racker and his group have reconstituted the NADH oxidase enzyme using liposomes containing cardiolipin (14); c. Cardiolipin levels are chloramphenicol sensitive (15) and d. Cardiolipin levels are decreased in the yeast cells in the repressed stages (15).

A series of experiments were carried out with the addition of cardiolipin, lecithin and total phospholipids in the *in vitro* integration systems and the results are given in Table 1. The data are indicative of an important (if not exclusive) role for cardiolipin in the anchoring of the proteins into the membrane.

Acknowledgement

J A is grateful to CSIR, New Delhi, for financial assistance.

References

1 Jayaraman,J., Cotman,C., Mahler,H.R. and Sharp,C.W. (1966) Arch. Biochem. Biophys. 116, 224

2 Dharmalingam,K. and Jayaraman,J. (1971) Biochem. Biophys. Res. Commun. 45, 1115

3 Chandrasekaran,K., Murugesh,N. and Jayaraman,J. (1978) Biochem. Biophys. Res. Commun. 62, 693

4 Chandrasekaran,K. and Jayaraman,J. (1978) FEBS Lett. 87, 52

5 Chandrasekaran,K., Dharmalingam and Jayaraman,J. Eur. J. Biochem. (1980) 103, 471

6 Green,D.E., Mil,S. and Kohout,P.M. (1955) J. Biol. Chem. 217, 551

7 Lowry,O.H., Rosebrough,N.J., Farr,A.L. and Randall,R.J. (1951) J. Biol. Chem. 193, 265

8 Bligh,E.C. and Dyer,W.J. (1959) Can. J. Biochem. Physiol. 37, 911

9 Fang,M. and Marinetti,G.V. (1969) In 'Methods in Enzymology' Vol.14. pp 598

10 King,E.J. and Wooten,I.D.P. (1959) In 'Microanalysis in clinical biochemistry, pp 77, Churchill, London

11 Fiske,C.H. and Subbarow,Y. (1925) J. Biol. Chem. 66, 375-400

12 Murugesh,N. and Jayaraman,J. (1975) Indian J. Biochem. Biophys. 12(1), 67

13 Singer,J. (1975) In 'Cell membranes' (Ed. Gerald Weissmann & Robert Claiborne) pp 35 H.P. Publishing Co., Inc. New York

14 Ragan,C.E. and Racker,E. (1973) J. Biol. Chem. 248, 2563

15 Murugesh,N. (1976) Ph.D Thesis submitted to Madurai Kamaraj University, Madurai, India