NUCLEAR AND CYTOPLASMIC GLUTAMATE DEHYDROGENASES (NADP-DEPENDENT) IN SACCHAROMYCES CEREVISIAE

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SUMMARY

The intracellular localization of NADP-dependent glutamate dehydrogenase has been studied in Saccharomyces cerevisiae.

Beside cytoplasmic GDH, enzyme activity has been found to be associated with the nuclear fraction in amounts comparable to those reported in nuclei of higher organisms.

The yield and distribution of both GDH activities have been analyzed in mutants showing, under particular growth conditions, defective mitochondrial functions.

INTRODUCTION

NADP-dependent glutamate dehydrogenase of yeast Saccharomyces cerevisiae has been described and characterized by several authors (1,2,3); the presence of NAD-dependent GDH, in lesser amounts, has also been reported (4). Although in higher organisms this enzyme was commonly believed of mitochondrial localization, GDH of yeast has often been found in the soluble cytoplasmic fractions (5,6). Although in the latter organism the enzyme may be very weakly bound to mitochondria, it may indeed have a different localization.

Several investigators have recently found another GDH associated with the purified nuclear fraction from animal tissue (7-13). We have extended the investigation to yeast, and the experiments reported below demonstrate that also yeast nuclei contain a relatively high GDH level. The purification and characterization of yeast nuclear GDH will be reported elsewhere (14): it shows properties that clearly differentiate it from the cytoplasmic enzyme.

We have investigated the possibility that the synthesis of the cytoplasmic and nuclear enzymes is regulated in dependence of the physiological conditions of the cells, using mutants of Saccharomyces cerevisiae defective in the mitochondrial functions.

EXPERIMENTAL

Strains: The following yeast strains of Saccharomyces cerevisiae were used: pts mutant strain 1511 (15) and an isogenic derivative rho$^-$ 1511-1.

Growth conditions: Cultures were grown in YEP medium consisting of 1% Difco

yeast extract and 2% Difco peptone; 1% glucose and 3% glycerol were added to the medium. Cultures were grown at 23°C and 36°C with vigorous shaking.

Respiration measurements: The oxygen uptake of the whole cells was measured polarographically with a KM Gilson Oxigraph.

Preparation of protoplasts: Early stationary phase cells were treated according to the procedure of Kováč (16) for preparation of yeast mitochondria, except that snail gut juice was substituted by Glusulase. When protoplasts were formed in 80% yield, they were disrupted in a Sorvall Omni Mixer for 30 sec. at the maximum speed without previously washing away the Glusulase. In fact, it was observed that GDH was partially extracted from the protoplasts by the lytic enzyme, which however did not interfere with the GDH assay.

Enzyme assay: The enzyme activity of the homogenate was assayed spectrophotometrically at 30°C, in cuvettes of 1-cm light path, by recording the decrease in absorbance at 340 nm of the reduced coenzyme. In a final volume of 1 ml, the mixture contained: 0.1 M $(NH_4)_2SO_4$, 0.1 M phosphate buffer pH 7.0, 0.013 M α-ketoglutarate and 0.1 mM NADPH. Activity is expressed as μmoles of cofactor oxidized per minute, using a molar extinction coefficient of 6.22×10^3 (17), one μmole representing one activity unit.

Reagents: Erythromycin used as lactobionate was from Abbott, Glusulase from Endo Lab., NADPH from Calbiochem, α-ketoglutarate from Pierce Chemicals. All the other reagents were of the highest purity commercially available.

RESULTS AND DISCUSSION

Nuclear gene mutations as well as cytoplasmic mutations can affect the mitochondrial functions in yeast (18,19). However, cells can grow on fermentable substrates using the glycolytic pathway. The same phenotype can be obtained by growing yeast cells in the presence of erythromycin (20). For the experiments reported below, we have used yeast strains and growth conditions which give rise to respiratory-deficient phenotypes.

The 1511 pts mutant strain, grown at 23°C and used as control, develops structurally and functionally unaffected mitochondria. After prolonged growth at 36°C, the mitochondria are altered and the "petite" phenotype is expressed (15,21).

The GDH level was measured in homogenates of cells grown at 23°C and 36°C for about 20 generations. After centrifugation of the homogenate at 800 X g, 85-90% of the activity was found in the supernatant; the remaining 10-15% was associated with the precipitate which contained nuclei, cell debris and a few intact cells. The activity remained in the pellet, even after repeated washing in 0.32 M sucrose, until the supernatant was completely devoid of enzymatic activity. After further purification of the nuclear fraction by high-speed centrifugation in 2 M sucrose (22), the activity was still bound to the nuclei and could be extracted by ultrasonic treatment (14). The results of these experiments are shown in Table 1. The yield of enzyme per gram of cells grown at 36°C was twice that of the control at 23°C, but the

Table 1

Distribution of GDH activity in cultures of 1511 strain grown at 23°C and 36°C

fraction	23°C (1.3 g cells)		36°C (1.8 g cells)	
	units/g	%	units/g	%
homogenate	0.535	100	1.240	100
800 x g supernatant	0.480	89.7	1.116	89.8
800 x g sediment	0.054	10.3	0.126	10.2

percentage of activity associated with the nuclear fraction was constant.

Table 2 shows the results obtained with cultures of the 1511 strain, grown for about 20 generations at 23°C in the presence and absence of 2.5 mg/ml erythromycin, and with cultures of the spontaneous rho$^-$ mutant strain 1511-1 grown at the same temperature. The respiratory-deficient phenotype is completely expressed in these cases and the mitochondrial functions, in terms of respiration, mitochondrial protein synthesis and cytochrome content, are blocked (21). The enzyme content in the homogenate, as well as the distribution between the nuclear and cytoplasmic fractions, were comparable to those found in cultures of 1511 strain grown at 36°C: also in these cases, the amount of GDH per gram of cells was two-fold higher than that of the control at 23°C.

The observation that cells with altered mitochondrial functions synthesize higher levels of both nuclear and cytoplasmic GDH could be explained by hypothesizing that a regulatory protein, synthesized in the mitochondria, controls the enzyme synthesis at the cytoplasmic level and that this function is lost under the conditions outlined above.

Further experiments were carried out in an attempt to clarify the localization of the activity, not associated with nuclei, which, in our experiments, was constantly found in the cell sap. An alternative method of preparation of mitochondria (23) was used, involving cell fragmentation by means of glass beads. Again, GDH activity was not found associated with the mitochondrial pellet, but was recovered in the soluble cytoplasmic fraction.

Table 2

Distribution of GDH activity in cultures of 1511 strain grown at 23°C in the presence and absence of erythromycin, and in cultures of the isogenic derivative rho⁻ 1511-1

fraction	control (2 g cells)		+erythr. (0.85 g cells)		rho⁻ (1.3 g cells)	
	units/g	%	units/g	%	units/g	%
homogenate	0.592	100	1.168	100	1.046	100
800 x g supernatant	0.536	90.5	1.040	89.2	0.968	83
800 x g sediment	0.056	9.5	0.126	10.8	0.184	17

This result, together with the finding that mutants with impaired mitochondrial functions do not show enzyme levels lower than the control, supports the conclusion reached by other investigators that the enzyme not associated with nuclei is localized in the soluble cytoplasmic phase and not in the mitochondria.

The biological role of nuclear GDH is still unclear. The isolation of suitable mutants, with altered nuclear GDH activity, could provide an effective tool to tackle this problem.

REFERENCES

1. Grisolia, S., Quijada, C.L., and Fernandez, M. (1964) Biochim. Biophys. Acta 81, 61-70.
2. Fourcade, A. (1968) Bull. Soc. Chim. Biol. 50, 1671-1679.
3. Fourcade, A., and Venard, R. (1971) Biochim. Biophys. Acta 242, 331-344.
4. Bernhardt, W., Zink, M., and Holzer, H. (1966) Biochim. Biophys. Acta 118, 549-555.
5. Perlman, Ph. S., and Mahler, H.R. (1970) Arch. Biochem. Biophys. 136, 245-259.
6. Hollenberg, C.P., Riks, W.F., and Borst, P. (1970) Biochim. Biophys. Acta 201, 13-19.
7. di Prisco, G., Banay-Schwartz, M., and Strecker, H.J. (1968) Biochem. Biophys. Res. Commun. 33, 606-612.
8. Zbarsky, I.B., Pokrovsky, A.A., Perevoshchikova, K.A., Gapparov, M.M., Lashneva, N.V., and Delektorskaya, L.N. (1968) Dokl. Akad. Nauk SSSR (Biochemistry) 181, 993.

9. di Prisco, G., and Strecker, H.J. (1970) Eur. J. Biochem. 12, 483-489.

10. Herzfeld, A., Federman, M., and Greengard, O. (1973) J. Cell Biol. 57, 475-483.

11. Franke, W.W., Deumling, B., Ermen, B., Jarasch, E.D., and Kleinig, H. (1970) J. Cell Biol. 46, 379-395.

12. Kato, T., and Lowry, O.H. (1972) J. Biol. Chem. 248, 2044-2048.

13. di Prisco, G., and Garofano, F. (1974) Biochem. Biophys. Res. Commun. 58, 683-689.

14. Camardella, L., di Prisco, G., Garofano, F., and Guerrini, A.M. (in preparation).

15. Puglisi, P.P., and Cremona, T. (1970) Biochem. Biophys. Res. Commun. 39, 461-469.

16. Kováč, L., Bednárová, H., and Greksák, M. (1968) Biochim. Biophys. Acta 153, 32-42.

17. Horecker, B.L., and Kornberg, A. (1948) J. Biol. Chem. 175, 385-390.

18. Ephrussi, B. (1953) Nucleo-Cytoplasmic Relations in Microrganisms. London, Oxford University Press.

19. Sherman, F. (1963) Genetics 48, 375-385.

20. Clark-Walker, G.D., and Linnane, A.W. (1967) J. Cell Biol. 34, 1-14.

21. Guerrini, A.M., Pedrini, M.A., and Gionti, E. (in preparation).

22. Rozijn, Th. H., and Tonino, G.J.M. (1964) Biochim. Biophys. Acta 91, 105-112.

23. Del Giudice, L., and Puglisi, P.P. (1974) Biochem. Biophys. Res. Commun. 59, 865-871.